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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.

3. Gaston M. The psychological care of patients following a myocardial infarction [Online]. 2003; Available from: URL: http://www.nursingtimes.net/the-psychologicalcare-of-patients-following-a-myocardialinfarction/199464.article/

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Study of antioxidant activity of sheep visceral protein hydrolysate: Optimization using response surface methodology

Nasim Meshginfar[(1)], **Alireza Sadeghi-Mahoonak**[(2)], **Aman Mohammad Ziaiifar**[(3)], **Mohammad Ghorbani**[(2)], **Mahdi Kashaninejad**[(2)]

Original Article

Abstract

BACKGROUND: The main objective of this experiment was optimal use of none edible protein source to increase nutritional value of production with high biological function, including antioxidant activity.

METHODS: Sheep visceral (stomach and intestine) was used as substrate. Response surface methodology (RSM) was used to optimize hydrolysis conditions for preparing protein hydrolysate from the sheep visceral, using alcalase 2.4 l enzyme. The investigated factors were temperature (43-52 °C), time (90-180 min), and enzyme/substrate ratio [60-90 Anson-unit (AU)/kg protein] to achieve maximum antioxidant activity. Experiments were designed according to the central composite design.

RESULTS: Each of the studied variables had a significant effect on responses ($P < 0.05$). Optimal conditions to achieve antioxidant activity were, temperature (48.27 °C), time (158.78), min and enzyme/substrate ratio (83.35) Anson-unit/kg protein. Under these conditions, antioxidant activity was 68.21%, R^2 for model was 0.983. The values indicated the high accuracy of the model to predict the reaction conditions considering different variables. The chemical analysis of protein hydrolysate showed high protein content (83.78%) and low fat content (0.34%).

CONCLUSION: Our results showed that protein hydrolysate of sheep visceral, can be used as a natural antioxidant with high nutritional value.

Keywords: Antioxidant Peptides, Protein Hydrolysate, Enzyme Hydrolysis, Optimization

Introduction

Sheep slaughter and edible and non-edible wastes with high content of protein, is considered as the waste of slaughter industry, include internal organs, specially digestive organs (including the stomach and the intestines), if this material is not transfer to refined section, will be suitable source to grow microorganisms. Furthermore, non-use of this material creates financial problems for industrial unit, the visceral amount obtained from sheep slaughter, is around 7-8% of total slaughter weight. In fact if the average weight of one sheep slaughter is around 32 kg. About 2.08 kg of waste will be converted.[1] Biological hydrolysis is the most proper method to produce products with high added value, like bioactive peptides.[2] Bioactive peptides have low molecular mass, which after entering the body are easily digested and absorbed. In fact, the protein absorption in the form of peptide sequences is well-absorbed into amino acids at cellular levels.[3] Bioactive peptides play a more important role in the inner biological conditions. The important functions of these compounds are anti-oxidation, anti-microbial, anti-cancer, and immune system enhancement activities. Antioxidant properties of this compound in vitro system are as well as in vivo system. The results of studies conducted on cardio-vascular diseases and a number of cancers, suggest the existence of an inverse relationship between antioxidant nutrients and progression of these diseases,[3] the necessity of using the natural antioxidants is one of the reason to produce this

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product. The precise mechanism of these antioxidant peptides is not clear, according to different conducted researches; these compounds effectively prevent lipid oxidation, and shows special effort in controlling and scavenging free radicals and metal ions.[4] The antioxidant activity of these compounds is particularly influenced by compositional of the constituent amino acids. Other important and influential factors of bioactive antioxidant peptides related to the amino acid sequences in the peptides, and the special structure of peptides, the reaction condition, type of protease used, and the degree of hydrolysis.[5,6] Several researches were performed to produce hydrolysate protein with antioxidant properties from animal sources.[7-9] Hydrolysate protein is now being used to synthesize medicinal formulations and food formulations as a specific compound with desired characteristic. This product has still good potential for technological and nutritional researches.[10]

This research aimed to produce protein-rich food with high nutritional value, using enzymatic hydrolysis process and evaluate the effects of manufacturing conditions (temperature, time, and amount of enzyme) on product characteristics (antioxidant activity).

Materials and Methods

Initial sample preparation
Sheep visceral was purchased from the city of Gorgan's local slaughterhouses. These raw materials were first completely washed by the high pressure water and kept in −25 °C until the beginning of the test.

To perform the test on the frozen materials, first they were placed in the refrigerator at 4 °C for defrost process, and they were cut into little pieces, then, they were grinded by a meat grinder and eventually the size of the pieces was decreased as much as possible. Grinded and minced mixture was immediately transferred by special containers to the autoclave and was sterilized for 15 min at 121 °C. After cooling at room temperature, the sterilized mixture homogenized by a mixer as much as possible and then centrifuged at 6000 × g for 30 min at 4 °C. After centrifugation, the material was divided into three phases, the upper phase included lipid (fat), the middle phase, water, and at last, the enriched and deposited protein was accumulated in the lowest phase. The protein content with low level of fat was collected for further tests.[11]

The preparation of hydrolysate protein
All hydrolysis reactions were performed in 100 ml

Erlenmeyer flasks, containing 20 g of protein samples (proteins without any fat) with Tris-HCl buffer of 1:2 (w/v).[12] The alcalase enzyme (from Bacillus licheniformis with a proteolytic activity of 2.4 (AU/ml) (one Anson unit [AU] is defined as the amount of enzyme that will release 1.0 mEq of tyrosine from urea-denatured hemoglobin/min at 25 °C, pH 7.5) was added to the mixture at pH 8 (pH was adjusted with the buffer and was also suitable for the enzyme activity and helped to the stabilization of pH during the process). All reactions were performed in a shaking incubator (Vision, Scientific Co., Ltd.) with constant agitation (200 rpm). At the end of experiment, the enzyme activity was finished by heating the mixture in a water bath at 85° C for 20 min.[13] The mixture's temperature was decreased by using ice-bath, then and centrifuged at 6700 × g for 20 min at 10 °C, for the purpose of collecting the surface liquid.[13] The supernatant was dried using the freeze dryer. Hydrolysate protein production for each treatment was performed in three replications.

Measuring antioxidant activity
1-2,2-Diphenyl-1picrylhydrazyl free radical scavenging assay
For this purpose 1000 μl from each sample with 1000 μl 1-2,2-diphenyl-1picrylhydrazyl (DPPH) (0.1 Mm) produced in 99.5% ethanol were mixed in test tubes. Test tubes were vigorously stirred for 2 min. The mixture was then placed in the room temperature and kept in dark, and then the amount of radical DPPH absorption in 517 nm was measured.[6] It should be noted that, in the control sample instead of using hydrolyzed protein, 1000 μl of ethanol was used. In this experiment, butylated hydroxy toluene (BHT) at a concentration of 0.02 mg/ml was used for comparison.

Chemical analysis
Moisture content was determined by placing approximately 2 g of sample into a pre-weighted aluminum dish. Samples were then heated in an oven at 105 °C until a constant weight.[14] The total crude protein (N × 6.25) in raw materials and protein hydrolysate (liquid and powder) was determined using the Kjeldahl method.[14] Total lipid in samples was determined by Soxhlet extraction.[14] Ash content was determined by heating a pre-burning sample in an electric furnace at 600 °C until a white ash was formed.[14]

Statistical analysis
In this study, the Minitab for Windows (version 16; Minitab Inc., State College, PA, USA) and response surface methodology (RSM) was used to optimize the production condition of hydrolysate protein. RSM

with central composite design and three variables, six replicates at the central point, without block and alpha = 1.414 was considered for this test. Three variables of temperature (X_1), time (X_2), and enzyme ratio (X_3) are shown in table 1.

Results

Chemical analysis

The results related to the chemical compound of the raw material, defatted row material, and protein hydrolysate are presented in table 2.

DPPH free radical scavenging assay

The antioxidant activity was considered as the response variable and was presented in table 3. The effect of each independent variable was eventually examined on the surface of this response. The model proposed for the response is presented in Equation 1:

$$Y_1 = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_{ii}^2 + \sum_{i=1}^{2} \sum_{j=1}^{3} \beta_{ij} x_i x_j \quad (1)$$

The results of the effect of each variable on the response and model evaluation are presented in table 4.

3D surface plots and contour plots in order to impact of temperature (X_1), time (X_2), enzyme to substrate ratio (X_3) on response are presented in figure 1.

Table 1. Independent factors, their coded, and actual levels used in the experiment

Factor	Levels				
	+α	+1	0	−1	−α
Temperature (°C, X_1)	53.86	52	47.5	43	41.13
Time (min, X_2)	198.60	180	135.0	90	71.37
Enzyme ratio (AU/kg protein, X_3)	96.21	90	75.0	60	53.79

α: 1.414; X_1: Temperature; X_2: Time; X_3: Enzyme ratio

Table 2. Proximate composition (%) of raw materials and protein hydrolysate base on dried weight

Material	Protein	Fat	Moisture	Ash
Fresh viscera	10.30 ± 2.04	4.14 ± 1.04	84.50 ± 1.35	0.13 ± 0.05
Partially defatted visceral	22.55 ± 1.06	2.38 ± 1.45	72.50 ± 1.52	0.17 ± 0.01
Protein hydrolysate	83.78 ± 1.34	0.34 ± 1.03	8.61 ± 0.85	7.05 ± 0.08

All values are means of triplicate determinations (mean ± SD); SD: Standard deviation

Table 3. Experimental design used in response surface methodology studies by using three independent variables showing observed 1-2,2-diphenyl-1picrylhydrazyl free radical

Run no.	X_1	X_2	X_3	DPPH radical scavenging (%)
1	43.00	90.00	60.00	34.00
2	52.00	90.00	60.00	46.32
3	43.00	180.00	60.00	34.20
4	52.00	180.00	60.00	36.70
5	43.00	90.00	90.00	31.93
6	52.00	90.00	90.00	54.12
7	43.00	180.00	90.00	42.31
8	52.00	180.00	90.00	55.21
9	41.13	135.00	75.00	22.35
10	53.86	135.00	75.00	36.60
11	47.50	71.38	75.00	61.63
12	47.50	198.63	75.00	63.89
13	47.50	135.00	53.79	49.50
14	47.50	135.00	96.21	60.80
15	47.50	135.00	75.00	66.13
16	47.50	135.00	75.00	66.17
17	47.50	135.00	75.00	68.17
18	47.50	135.00	75.00	66.30
19	47.50	135.00	75.00	67.50
20	47.50	135.00	75.00	64.62

X_1: Temperature; X_2: Time; X_3: Enzyme ratio; DPPH: 1-2,2-Diphenyl-1picrylhydrazyl

Table 4. ANOVA table for response as affected by independent variables during optimization experiments

Factors	df	Regression coefficient	P
Model	9	−2077.400	< 0.001
Variables			
X_1	1	84.880	< 0.001
X_2	1	0.470	< 0.010
X_3	1	−1.145	< 0.010
Interaction			
$X_1 \cdot X_2$	1	−0.010	< 0.001
$X_1 \cdot X_3$	1	0.040	< 0.001
$X_2 \cdot X_3$	1	0.001	< 0.001
Quadratic effect			
X_1^2	1	−0.890	< 0.001
X_2^2	1	−0.001	< 0.001
X_3^2	1	−0.102	< 0.001
Lack of fitness	5		0.436
R^2-Pred		0.983	
R^2-Adj		0.992	

Df: Degree of freedom; X_1: Temperature; X_2: Time; X_3: Enzyme ratio; X^2: X squared; R^2: Two factors of the regression equations; R^2-Pred: Predicted r-square; R^2-Adj: Adjusted r-square

a₁

a₂

b₁

b₂

c₁

c₂
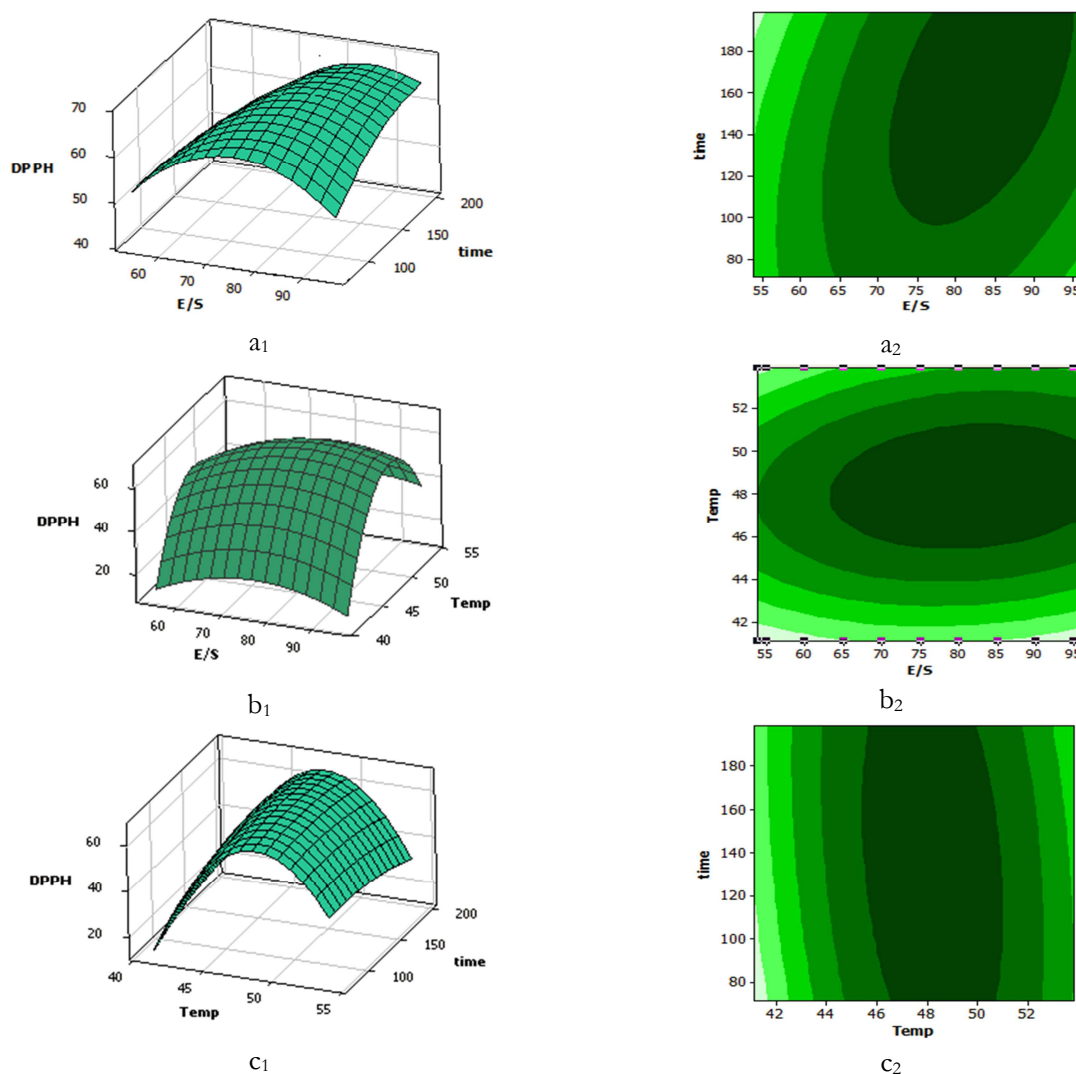
Figure 1. Response surfaces and contour plots for the effect of variables on 1-2,2-diphenyl-1picrylhydrazyl (DPPH) as a function of different hydrolyzing conditions: time and enzyme activity (a), temperature and enzyme activity (b), time and temperature (c)

Discussion

Chemical analysis

These results showed that the highest amounts of protein in hydrolysate protein, defatted raw material, and the fresh material are respectively 83.78 ± 1.34%, 22.55 ± 1.06%, and 10.30 ± 2.04% (based on the dry mass). The results of other researchers also suggest the high amount of protein in hydrolysate protein.[10,15] The amount of fat in the raw material is 4.14 ± 1.04% (based on the dried mass). This amount was drastically decreased after the defatted and separation from the protein (P < 0.05). It should be noted that the amount of fat in the hydrolysate protein was also greatly decreased (0.34 ± 1.03%, P < 0.05). This could due to release of fat and its sediments along with non-soluble proteins during the high-speed centrifuge.[7,15] Furthermore, some of the fat was seen as a separate layer after centrifuging process on supernatant. The hydrolysate protein is identified as a low fat product. The results of other researchers suggest that the amount of fat in hydrolysate protein is often less than 1%.[10,15]

DPPH free radical scavenging assay

The ANOVA table showed that every variable factor had significant effect on response (P < 0.05). The relation between antioxidant activity and hydrolysis parameters was quadratic. The amount of R[2] for the provided model was achieved with high values (0.992), which suggest the good ability of this model to predict the reaction conditions. The results also show that model's lack of fitness with experimental data was not significant (0.436) (P > 0.05), which suggest the suitability of the model for the test data.

Surface response graph showed the effect of two variables, whereas the third variable was placed at middle level. The ≤ highest amount of antioxidant activity achieved with applying a temperature: 45 °C and relatively high amount of enzyme (greater than 65 °C), which in these situations the effect of time on the activity rate of free radical is less significant compared to others factors. In fact by creating suitable conditions of temperature and the enzyme content, it is possible to achieve suitable antioxidant activity.

According to the charts-related to the effect of enzyme-time (a) and enzyme-temperature (b), the suitable range of this variable for creating acceptable antioxidant activity has been set between 65 and 95 AU/kg proteins, which tend to achieve the highest amount of antioxidant activity. The chart related to enzyme- time effect also show the amount of enzyme used in the test should be increased, when

time of process is increased. The temperature had the most important effect on antioxidant activity rate. Chabeaud et al. in a study on the optimization of antioxidant activity of the hydrolysate fish protein of Saithe, reported that the highest rate of antioxidant activity (66.4%), at 60 °C temperature, pH 8, enzyme to substrate ratio of 8.53 % (AU/kg protein), is achieved after 10.8 min of hydrolysis. These researchers also referred to the effect of peptide properties in antioxidant activity.[16] In another study conducted by Taheri et al. on improving antioxidant activity of hydrolysate sardine protein, the optimal conditions included temperature higher than 45 °C, time between 80 and 120 min, and a moderate range of enzyme. The researchers also reported the improvement of hydrolysis process results in the release of antioxidant peptides from protein chain, but continuing the process of hydrolysis could decrease this activity instead.[12]

Optimizing antioxidant activity and evaluating the validity of model

The optimized conditions for hydrolysate protein antioxidant activity were predicted. These conditions include the temperature of 48.27 °C, time 158.78 min, and enzyme to substrate ratio of 83.35 AU/kg protein, which 67.98% represents free radical scavenging activity. In order to evaluate the statistical model validity, an extra test was carried out under the mentioned conditions and the free radical scavenging activity was estimated as 68.21%. This result shows that the predicted free radical scavenging amount by presented model, is compatible with the amount achieved in the experiment. These conditions represent that the model could appropriately show the effect of three variables such as temperature, time, and enzyme/substrate ratio DPPH free radical scavenging activity.

Conclusion

In the current study, in addition to producing hydrolysate protein products, the focus shifted to examine the effect of different condition on the current feature and its optimization. The results of protein hydrolysis of the antioxidant activity optimization showed that the ideal amount achieved at 48.27 °C, during 158.78 min, and enzyme to substrate ratio of 83.35 AU/kg protein. The highest amount for the antioxidant activity of this product was estimated 68.21%. Temperature had more effect on the antioxidant activity of product in comparison with the other two variables (P < 0.05).

The antioxidant behavior of hydrolysate protein was well under the influence of the chemical structure and properties of the peptides. The result of current study shows that the protein hydrolysate of sheep visceral could be considered as a natural antioxidant, instead of synthetic antioxidant.

Acknowledgments

Conflict of Interests

Authors have no conflict of interests.

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P-wave dispersion and its relationship to aortic stiffness in patients with acute myocardial infarction after cardiac rehabilitation

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Original Article

Abstract

BACKGROUND: The aim of our study was to investigate the P-wave dispersion from standard electrocardiograms (ECGs) in patients with acute myocardial infarction (AMI) after cardiac rehabilitation (CR) and determine its relation to arterial stiffness.

METHODS: This is a prospective study included 33 patients with AMI and successfully re-vascularized by percutaneous coronary intervention (PCI) underwent CR. Left ventricular ejection fraction (LVEF) was measured by biplane Simpson's method. Left atrium (LA) volume was calculated. The maximum and minimum durations of P-waves (Pmax and Pmin, respectively) were detected, and the difference between Pmax and Pmin was defined as P-wave dispersion (Pd = Pmax–Pmin). Aortic elasticity parameters were measured.

RESULTS: LVEF was better after CR. The systolic and diastolic blood pressures decreased after CR, these differences were statistically significant. With exercise training, LA volume decreased significantly. Pmax and Pd values were significantly shorter after the CR program. The maximum and minimum P-waves and P-wave dispersion after CR were 97 ± 6 ms, 53 ± 5 ms, and 44 ± 5 ms, respectively. Aortic strain and distensibility increased and aortic stiffness index was decreased significantly. Aortic stiffness index was 0.4 ± 0.2 versus 0.3 ± 0.2, P = 0.001. Aortic stiffness and left atrial volume showed a moderate positive correlation with P-wave dispersion (r = 0.52, P = 0.005; r = 0.64, P < 0.001, respectively).

CONCLUSION: This study showed decreased arterial stiffness indexes in AMI patient's participated CR, with a significant relationship between the electromechanical properties of the LA that may raise a question of the preventive effect of CR from atrial fibrillation and stroke in patients with acute myocardial infarction.

Keywords: Cardiac Rehabilitation, P-Wave Dispersion, Aortic Stiffness, Acute Myocardial Infarction

Introduction

Increases in the P-wave dispersion from standard electrocardiograms (ECGs) with subsequent development of atrial fibrillation (AF) have been identified in patients with a wide range of cardiovascular disorders.[1] AF is the most common arrhythmia treated in clinical practice and approximately 33% of arrhythmias related hospitalizations are for AF. It is associated with a fivefold increase in the risk of stroke and two-fold increase in the risk of all-cause mortality.[2] The assessment of left atrium (LA) mechanical and electromechanical functions are accepted as risk factors of AF. Among the noninvasive and invasive methods to evaluate the inter-atrial conduction, the basic and the most frequently used one is the electrocardiographic P-wave dispersion.[3]

However, prolonged inter-atrial conduction time (IACT) is associated with the development of atrial fibrillation and abnormal LA function.[4-6] The prolongation of electromechanical delay (EMD) and the inhomogeneous propagation of sinus impulses are well-known electrophysiological characteristics of the atria prone to fibrillation.[7] IACT can be measured by two-dimensional (2D)-Doppler echocardiography, including tissue Doppler imaging. IACT measured by 2D-Doppler echocardiography and its association with indices of

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LA function has been reported in a few studies in patients with left ventricular (LV) systolic dysfunction.[8-11] Deniz et al. compared the tissue Doppler echocardiography and electrophysiological study in the measurement of atrial conduction times and found a moderate correlation between intra-left atrial conduction time by echocardiography (ILCT-echo) and ILCT by electrophysiology (ILCT-eps), which means that tissue Doppler echocardiography can be used to evaluate atrial conduction time.[12]

In recent times, aortic stiffness was found to influence the diameter of the LA and expose the patient to embolic stroke by increasing their risk of atrial fibrillation (AF). Previous studies revealed an inverse relationship between aortic distensibility and cardiovascular risk factors.[13-15]

Benefits of cardiac rehabilitation (CR) for patients with cardiovascular diseases have been shown by many clinical investigators.[16-18] The effect of CR on total mortality was independent of coronary heart disease diagnosis, type of CR, dose of exercise intervention and length of follow-up.[19] Comprehensive CR program includes not only exercise training, also diet counselling, weight control management, lipid management, smoking cessation, blood pressure monitoring, and psychosocial management that aims to optimize cardiovascular risk reduction.

The aim of our study was to investigate P-wave dispersion from standard ECGs and determine its relation to arterial stiffness in patients with acute myocardial infarction (AMI) after CR.

Materials and Methods

Study design
This is a prospective study included 33 patients with AMI and successfully re-vascularized by percutaneous coronary intervention (PCI) underwent CR between October 2012 and April 2013. Each patient had performed intensive out-patient CR program (also known as Phase II CR) for 5 times a week during 6 weeks at the CR center of our education and research hospital. All patients were asymptomatic and had been in a clinically stable condition after discharge period. Lower-risk patients following an acute cardiac event enrolled this study. High risk patients with severe residual angina, severe ischemia, poorly controlled hypertension, hypertensive or any hypotensive systolic blood pressure response to exercise and unstable concomitant medical problems (diabetes prone to hypoglycemia) were excluded from the study. During the training, ECGs were continuously telemonitored. Typical training in CR started with 5 min warm up, followed with 20 min aerobic training and 10-15 min cool down.

This study complied with the Declaration of Helsinki, was approved by the local Ethical Committee and written consent was obtained from each patient before CR.

Electrocardiographic evaluation of atrial conduction
Standard ECG were taken from all patients with sweeping rate of 50 mm/s and amplitude of 1 mV/cm. P-wave durations was measured manually in all simultaneously recorded 12 leads of the surface ECG. The mean P-wave duration for at least three complexes was calculated in each lead. The onset of the P-wave was defined as the point of first visible upward slope from baseline for positive waveforms, and as the point of first downward slope from baseline for negative waveforms. The return to the baseline was considered the end of the P-wave. The Pmax measured in any of the 12 leads of the surface ECG was used as the longest atrial conduction time. The maximum and minimum durations of P-waves (Pmax and Pmin, respectively) were detected, and the difference between Pmax and Pmin was defined as P-wave dispersion (Pd = Pmax–Pmin).

Echocardiography
A Vivid 7 ultrasound system (GE Vingmed Ultrasound, Horten, Norway) was used, and all images and measurements were acquired from the standard views, according to the guidelines of the American Society of Echocardiography. LV end-diastolic volume was measured, and left ventricular ejection fraction (LVEF) was calculated by the Simpson method by apical four-chamber view.

LA maximum anterio-posterior diameter (D1) was measured in the parasternal long-axis views. LA superior-inferior diameter (D2) was measured from the mitral annular plane to the posterior wall of the LA, and medial-lateral diameter (D3) was measured in the apical 4-chamber view (LA volume was calculated with the formula; $D1 \times D2 \times D3 \times 0.523$).

Tissue Doppler echocardiography was performed by transducer frequencies of 3.5-4.0 MHz, adjusting the spectral pulsed Doppler signal filters until a Nyquist limit of 15-20 cm/s and using the minimal optimal gain. In the apical four-chamber view, the pulsed Doppler sample volume was placed in order at the level of LV lateral mitral annulus, septal mitral annulus, and right ventricular tricuspid annulus. Atrial electromechanical coupling (PA), the time interval from the onset of the P-wave

on the surface ECG to the beginning of the late diastolic wave (Am); was obtained from the lateral mitral annulus (PAlat), septal mitral annulus (PAsep), and tricuspid annulus (PAtricus). The difference between PAlat and PAtricus was defined as the inter-atrial EMD, while the difference between PAsep and PAtricus was defined as the intra-atrial EMD. Every effort was made to align the pulsed wave cursor that the Doppler angle of incidence was as close to 0 as possible to the direction of these walls. All participants in our study also showed no clinical evidence of pulmonary hypertension, and systolic pulmonary artery pressure estimated by Doppler echocardiography was < 35 mmHg.

Systolic and diastolic ascending aortic diameters were measured on M-mode tracings at 3 cm above the aortic valve. An average of three beats was analyzed M-mode traces were recorded at a speed of 50 mm/s and Doppler signals were recorded at a speed of 100 mm/s. Simultaneous electrocardiographic recordings were also taken. Systolic diameter was measured at the maximal anterior motion of the aorta, while diastolic diameter was measured at the peak of the QRS complex on the simultaneous ECG.

Aortic elasticity parameters were calculated using the following formulas:

Aortic strain (%) = (aortic systolic–diastolic diameter) × 100/aortic diastolic diameter

Aortic stiffness index = (systolic/diastolic blood pressure)/aortic strain

Aortic distensibility (cm²/dyne/10⁶) = 2 × aortic strain/ (systolic–diastolic blood pressure).

Statistical analyses

All values were expressed as a mean ± SD. Data were analyzed using the SPSS for Windows (version 15.0, SPSS Inc., Chicago, IL, USA) and considered as significant if P < 0.05. Statistical analysis was performed using Student's t-test. Linear regression and Pearson correlation analysis were used for correlation of variables of interest. P-value < 0.05 was considered to indicate statistical significance.

Results

Thirty-three participants in sinus rhythm after AMI and successfully revascularization by PCI were recruited into the study. The mean age of the patients was 57 years. Infarct related artery was left anterior descending in 13 patients, circumflex coronary artery in 6 patients, and right coronary artery in 14 patients. No changes were done in medical therapy of the patients during the follow-

up, and there were no complications or arrhythmia in subjects during the study period. Patient demographics and clinical characteristics are presented in table 1.

Table 1. Patient demographics and clinical characteristics (n = 33)

Patients	Value
Age (year)	57 ± 7 (mean ± SD)
Gender	
Male, n (%)	27 (81)
Female, n (%)	6 (19)
Diabetes, n (%)	13 (39)
Hypertension, n (%)	19 (57)
Hyperlipidemia, n (%)	14 (42)
Smoking, n (%)	15 (45)
IRA	
LAD	13 (40)
CX	6 (18)
RCA	14 (42)

SD: Standard deviation; IRA: Infarct related artery; LAD: Left anterior descending; CX: Circumferential coronary artery; RCA: Right coronary artery

LVEF was improved with CR (P < 0.001). In comparison with the baseline, Pmax, and Pd values were significantly shorter after the CR program (P = 0.001 and P = 0.019, respectively). Furthermore, mitral lateral EMD (PAlat), septum EMD (PAsep), and tricuspid EMD (PAtricus) were decreased with the CR. Calculated inter-atrial and intra-atrial EMD were significantly lower after the CR compared to the baseline (21 ± 5 vs. 18 ± 4 ms , P < 0.001; 6 ± 2 vs. 4 ± 2 ms, P < 0.001). LA volume was decreased with exercise based CR (P < 0.001). IACT and P-wave dispersion showed a moderate positive correlation with left atrial volume (r = 0.591; P < 0.001, r = 0.615; P < 0.001, respectively). P-wave measurements and atrial EMD parameters at baseline and after the CR are set out in table 2.

The systolic and diastolic blood pressures decreased after CR, these differences were statistically significant. Aortic strain and distensibility increased and aortic stiffness index was decreased significantly after CR (P = 0.001). Aortic stiffness showed a moderate positive correlation with P-wave dispersion (r = 0.52; P = 0.005). Aortic elasticity parameters and blood pressure measurements of patients before and after CR are represented in table 3.

Table 2. Electromechanical delay and P-wave dispersion before and after cardiac rehabilitation

Echocardiographic and ECG parameters	Before CR (mean ± SD)	After CR (mean ± SD)	P
LVEF (ejection fraction)	51.4 ± 9.9	54.6 ± 9.3	< 0.001
LA volume (ml)	34.0 ± 9.7	33.0 ± 8.5	< 0.001
Mitral lateral (PAlat) EMD (ms)	62.0 ± 7.7	57.0 ± 7.8	0.001
Septum (PAsep) EMD (ms)	47.0 ± 10.0	43.0 ± 8.0	0.049
Tricuspid (PAtricus) EMD (ms)	40.0 ± 9.0	39.0 ± 7.0	0.418
Intra-atrial EMD (ms)	6.0 ± 2.0	4.0 ± 2.0	< 0.001
Inter-atrial EMD (ms)	21.0 ± 5.0	18.0 ± 4.0	< 0.001
Pmax (ms)	102.0 ± 8.0	97.0 ± 6.0	0.001
Pmin (ms)	55.0 ± 5.0	53.0 ± 5.0	0.044
Pd (ms)	46.0 ± 5.0	44.0 ± 5.0	0.019

ECG: Electrocardiograms; CR: Cardiac rehabilitation; SD: Standard deviation; LVEF: Left ventricular ejection fraction; LA: Left atrium; PAlat: Lateral mitral annulus; PAsep: Septal mitral annulus; PAtricus: Tricuspid annulus; EMD: Electromechanical delay; Pmax: Maximum P-wave duration; Pmin: Minimum P-wave duration; Pd: P-wave dispersion

Table 3. The aortic elasticity parameters and blood pressure of the individuals before and after cardiac rehabilitation

Parameters	Before CR (mean ± SD)	After CR (mean ± SD)	P
Systolic BP (mmHg)	132.00 ± 14.00	123.00 ± 13.00	< 0.001
Diastolic BP (mmHg)	80.00 ± 11.00	75.00 ± 10.00	< 0.001
Aortic strain (%)	4.00 ± 2.00	6.00 ± 3.00	< 0.001
Aortic stiffness index	0.40 ± 0.20	0.30 ± 0.20	< 0.001
Aortic distensibility (cm2/dyne/106)	0.19 ± 0.10	0.28 ± 0.10	< 0.001

CR: Cardiac rehabilitation; SD: Standard deviation; BP: Blood pressure

Discussion

The patients in the present study were asymptomatic and did not have a history of AF. To the best of our knowledge, the relative contribution of arterial stiffness to the P-wave dispersion from standard ECGs in patients after CR as a risk of developing AF has not been evaluated. Increased P-wave dispersion has been reported in various clinical settings, including coronary artery disease, hypertension, rheumatic mitral stenosis, mitral annular calcification and hypertrophic cardiomyopathy.[20-24] The mechanism of P-wave dispersion prolongation in these patients is thought to be due to structural and electrophysiological changes in the atrial myocardium. Chronic elevation of LV filling pressures may cause atrial fibrosis contributing to the prolongation of atrial activation time.[25] Several studies have suggested that increased P-wave duration may be associated with myocardial ischemia, altered autonomic control, LV diastolic dysfunction, enlarged left atrial dimension, elevated left atrial pressure and fibrosis, and aortic elasticity.[26-32] Also, Emiroglu et al. demonstrated that prolonged EMD and Pd found in hypertensive patients could be related with increased incidence of atrial fibrillation.[33] After the CR program with exercise in this study, left atrial volume was improved, and the ECG-derived Pmax and Pd values were also decreased compared to the baseline, which may suggests the decrease in the incidence of long-term AF risk.

An increase in aortic stiffness may increase the risk of stroke through several mechanisms such as an increase in central pulse pressure or an increase in carotid intima-media thickness, promoting the development of atherosclerotic lesions and thus the likelihood of plaque rupture.[34-36] Potential mechanisms include the possibility that increased arterial stiffness predisposes to neurohormonal activation[37] or a generalized cardiovascular inflammatory response,[38] which, in turn may contribute to the development of AF.[39] Cross-sectional studies show strong correlations between elevated C-reactive protein (CRP) and aortic stiffness. Exercise training is associated with reduced CRP levels, which suggests that exercise training has anti-inflammatory effects on atherosclerosis therefore, aortic stiffness. Furthermore, aerobic exercise training regulates the neurohormonal activation by reducing sympathetic and enhancing parasympathetic (vagal) activity, as evidenced by increased heart rate variability and reduced baroreceptor sensitivity which suggests the decrease in aortic stiffness.[40]

As suggested by Gosse and Safar in view of a common embryological origin, the aorta may be considered along with the LA and ventricle as the third chamber of the left sided cardiac pump

transforming the systolic output of the left ventricle into a continuous flow.[41] Our findings support this theory; after CR in patients with AMI as the aortic stiffness and LA volume decreases, therefore EMD decreases as well.

The favorable impact of CR on aortic stiffness may contribute to the reduction of the extent of atherosclerosis, but also it may prevent the risk of the occurrence of AF. This study suggests that improvement in arterial stiffness may contribute to decrease the LA electromechanical dysfunction, namely, the risk of AF and stroke.

Limitations

The number of patients with CR reported in this investigation was small and female subjects were too few. Also, follow-up after CR in terms of the development of AF was lacking. In addition, we did not perform continuous Holter recordings; we could not be sure about clinically silent paroxysmal AF episodes. This study did not directly address the issue of a link between stiffness and AF and rather used P-wave dispersion as a surrogate marker of the risk of AF. Studies with larger sample size with group analysis of CR would be beneficial in further evaluating the role of CR as a protector from the risk of AF and stroke.

Conclusion

The present study demonstrates the decreased arterial stiffness indexes in AMI patient's participated CR, with a significant relationship between the electromechanical properties of the LA. Therefore, this study illustrates the importance of CR and reopens the question of a new potential benefit of CR in the prevention of AF and stroke in patients with AMI beyond increase in physiological well-being of the individuals.

Conflict of Interests

Authors have no conflict of interests.

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Antiatherogenic, hepatoprotective, and hypolipidemic effects of coenzyme Q10 in alloxan-induced type 1 diabetic rats

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Original Article

Abstract

BACKGROUND: Diabetes mellitus, one of the leading metabolic syndromes, accounts for highest morbidity and mortality worldwide. In this study, we examined possible protective effect of coenzyme Q10 on lipid profile, atherogenic index, and liver enzyme markers in alloxan-induced type 1 diabetic rats.

METHODS: A total of 30 male rats were randomly divided into three groups; group 1 as control, group 2 diabetic untreatment, and group 3 treatments with coenzyme Q10 by 15 mg/kg i.p. daily, respectively. Diabetes was induced in the second and third groups by alloxan injection subcutaneously. After 8 weeks, the levels of fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), high density lipoprotein (HDL), atherogenic index, atherogenic coefficient, cardiac risk ratio, and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) of all groups were analyzed. Data were analyzed using non-parametric Mann-Whitney test (using SPSS) and $P < 0.05$ was considered as significant.

RESULTS: Coenzyme Q10 inhibited significantly the activities of ALT (11.17%), AST (19.35%) and ALP (36.67%) and decreased FBG (21.19%), TG (37.24%), TC (17.15%), LDL (30.44%), VLDL (37.24%), atherogenic index (44.24%), atherogenic coefficient (49.69%), and cardiac risk ratio (37.97%), HDL level was significantly (33.38%) increased when treated with coenzyme Q10.

CONCLUSION: The findings of this study suggest that coenzyme Q10 exert beneficial effects on the lipid profile, atherogenic index, and liver enzymes activity in alloxan-induced type 1 diabetic rats.

Keywords: Diabetes, Lipid Profile, Atherogenic Index, Rats, Liver Enzymes, Coenzyme Q10

Introduction

Diabetes mellitus, one of the leading metabolic syndromes, accounts for highest morbidity and mortality worldwide.[1] Diabetes mellitus is characterized by abnormalities in carbohydrate, lipid and protein metabolism due to complete or relative insufficiency of insulin secretion from pancreatic β-cells and/or defect in insulin action.[2] Oxidative stresses is a state of imbalance between oxidant and antioxidant systems.[3]

In recent times, much attention has been focused on the central and key role of oxidative stress in the pathogenesis of different diabetic complications.[4] Several studies have shown that antioxidant treatment reduces diabetic complications.[5] Due to increasing demand of patients for the use of natural products with anti-

diabetic activity, the general trend now is to use the natural products for medicinal application in their natural available form.[6] Polyphenols, well-known antioxidants, have also been showed to function as anti-diabetic by reducing blood glucose levels.[7,8] Researchers are recently interested in investigation and research into extraction of natural antioxidants to replace synthetic antioxidants.[9,10] Therefore, the research into the determination of the natural antioxidant source is very important to promote public health.

Coenzyme Q10 is a natural human ubiquinone, and it has fundamental role in mitochondrial energy (adenosine triphosphate) production in the respiratory chain.[11,12] It plays a role in extra-mitochondrial redox activity in the cell membrane. Coenzyme Q10 is also antioxidant, scavenging free

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radicals, and inhibiting lipid peroxidation.[13,14] The antioxidant effect of coenzyme Q10 is greater than vitamin E.[15] Coenzyme Q10 is also known to enhance the availability of other antioxidants such as vitamin C, vitamin E, and β-caroten.[16]

Since the hypolipidemic, antiatherogenic, and liver protective effects of coenzyme Q10 in alloxan-induced type 1 diabetic rats have not previously been reported; the objectives of the present study were to investigate antiatherogenic, hepatoprotective, and hypolipdemic effects of coenzyme Q10 in alloxan-induced type 1 diabetic rats.

Materials and Methods

Experimental design
Animals
Thirty male mature Sprague-Dawley rats (180-200 g) were obtained from Pasteur Institute of Tehran, Iran, and were allowed to adapt themselves with the new location for 1 week. This study was approved by the Animal Ethics Committee of Lorestan University of Medical Sciences, Iran, with accordance to the national health and medical research council guidelines. The rats were randomly divided into three groups (10 per each). The studied groups were as follows: group 1 as control, group 2 as diabetic without treatment, and group 3 as diabetic treatment with coenzyme Q10 (C9538, Sigma Chemical Co., St. Louis, MO, USA).

Diabetes induction
Diabetes was induced after overnight fasting in the second and third groups by injection of alloxan monohydrate (120 mg/kg) subcutaneously.[17] β-Cell degradation by alloxan leads to release of more insulin. Because of acute hypoglycemia, the rats received 10% sucrose solution for 48 h instead of drinking water. Five days after induction of diabetes, blood samples were gathered from the end part of tails. Blood glucose was measured by glucometer and the rats with blood glucose level of ≥ 300 mg/dl (16.7 mmol/l) were considered as diabetic.[18] During the first 5 days after diabetes induction, 1-3 rats per group died because of alloxan toxicity. The rats were kept at 12/12 dark-light period in 21 ± 3° C temperature. All animals were allowed free access to food and water *ad libitum* during the experiment. The third group was treated with coenzyme Q10 by 15 mg/kg i.p. daily.[19]

The treatment was begun at the 1st day of diabetes induction. After 8 weeks treatment, animals were anesthetized (nesdonal 50 mg/kg, i.p.), blood samples were obtained from hearts and allowed to clot for 20 min in laboratory temperature and then centrifuged at 3000 rpm for 10 min for serum separation.[20,21]

Biochemical study
The serum levels of fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), high density lipoprotein (HDL), atherogenic index and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) of all groups were analyzed.

FBG, TC, and TG concentrations and ALT, AST, and ALP activity were measured by biochemical analyzer using commercial kits (Olympus AU-600, Tokyo, Japan). HDL was analyzed by a Pars Azmoon kit from Iran. LDL and VLDL were determined by calculation using the Freidewald equation.[22,23] The atherogenic index was determined by calculation using the Ikewuchi and Ikewuchi equation.[24]

Statistical analysis
All values are expressed as mean ± standard error of mean. The data were compared between groups by Mann-Whitney U test. Statistical analyses were performed using the SPSS for Windows (version 13, SPSS Inc., Chicago, IL, USA) software. P-value of < 0.05 was considered as statistically significant.

Results
The level of glucose in the untreated diabetic rats was significantly (4.48-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (21.19%) inhibit the increase of glucose in comparison with the untreated diabetic animals (Table 1) (P < 0.05). The level of TC in the untreated diabetic rats was significantly (1.49-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (17.15%) inhibit the increase of TC in comparison with the untreated diabetic animals (Table 1) (P < 0.05). The level of TG in the untreated diabetic rats was significantly (1.32-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (37.24%) inhibit the increase of TG in comparison with the untreated diabetic animals (Table 1) (P < 0.05). The level of LDL in the untreated diabetic rats was significantly (2.72-fold) higher than that of control animals. The treatment of diabetic animal with coenzyme Q10 could significantly (30.44%) inhibit the increase of LDL in comparison with the untreated diabetic

animals (Table 1) (P < 0.05). The level of VLDL in the untreated diabetic rats was significantly (1.32-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (37.24%) inhibit the increase of VLDL in comparison with the untreated diabetic animals (Table 1) (P < 0.05). The level of HDL in the untreated diabetic rats was significantly (1.23-fold) lower than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (33.38%) increase of HDL in comparison with the untreated diabetic animals (Table 1) (P < 0.05).

The level of atherogenic index (units) [log (TG/HDL-C)] in the untreated diabetic rats was significantly (1.39-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (44.24%) inhibit the increase of atherogenic index in comparison with the untreated diabetic animals (Table 2) (P < 0.05).

The level of atherogenic coefficient [(TC-HDL-C)/HDL-C] in the untreated diabetic rats was significantly (2.45-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (49.69%) inhibit the increase of atherogenic coefficient in comparison with the untreated diabetic animals (Table 2) (P < 0.05). The level of cardiac risk ratio (TC/HDL-C) in the untreated diabetic rats was significantly (1.83-fold) higher than that of control

animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (37.97%) inhibit the increase of cardiac risk ratio (TC/HDL-C) in comparison with the untreated diabetic animals (Table 2) (P < 0.05). The level of cardiac risk ratio (LDL/HDL-C) in the untreated diabetic rats was significantly (3.45-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (47.66%) inhibit the increase of cardiac risk ratio (LDL/HDL-C) in comparison with the untreated diabetic animals (Table 2) (P < 0.05).

The activity of ALP in the untreated diabetic rats was significantly (1.87-fold) higher than that of control animals. The treatment of diabetic animal with coenzyme Q10 could significantly (36.67%) inhibit the increase of ALP in comparison with the untreated diabetic animals (Figure 1) (P < 0.05). The activity of ALT in the untreated diabetic rats was significantly (1.30-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (11.17%) inhibit the increase of ALT in comparison with the untreated diabetic animals (Figure 2) (P < 0.05). The activity of AST in the untreated diabetic rats was significantly (1.83-fold) higher than that of control animals (P < 0.05). The treatment of diabetic animal with coenzyme Q10 could significantly (19.35%) inhibit the increase of AST in comparison with the untreated diabetic animals (Figure 3) (P < 0.05).

Table 1. Effect of coenzyme Q10 on fasting blood glucose, triglyceride, total cholesterol, low density lipoprotein, very low density lipoprotein, and high density lipoprotein in diabetic rats

Parameter	Control	Diabetic	Diabetic + coenzyme Q10
FBG (mg/dl)	79.09 ± 27.66[*]	354.02 ± 58.32	279.07 ± 45.00[*#]
TG (mg/dl)	110.00 ± 29.66[*]	145.00 ± 28.01	91.00 ± 27.78[*]
TC (mg/dl)	75.32 ± 13.33[*]	112.05 ± 26.31	92.83 ± 21.14[*#]
HDL (mg/dl)	32.52 ± 7.75[*]	26.42 ± 12.49	35.24 ± 7.32[*]
LDL (mg/dl)	20.80 ± 3.87[*]	56.63 ± 6.94	39.39 ± 9.94[*#]
VLDL (mg/dl)	22.00 ± 4.89[*]	29.00 ± 4.78	18.20 ± 3.72[*]

Values are represented as mean ± SEM coenzyme Q10; [*] Significant change in comparison with diabetic without treatment at P < 0.05; [#] Significant change in comparison with control at P < 0.05; SEM: Standard error of mean; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

Table 2. Effect of coenzyme Q10 on atherogenic index, atherogenic coefficient, and cardiac risk ratio in diabetic rats

Parameter	Control	Diabetic	Diabetic + coenzyme Q10
Atherogenic index [(units) log (TG/HDL-C)]	0.53 ± 0.06[*]	0.74 ± 0.06	0.41 ± 0.02[*#]
Atherogenic coefficient [(TC-HDL-C)/HDL-C]	1.32 ± 0.72	3.24 ± 0.87	1.63 ± 0.21
Cardiac risk ratio (TC/HDL-C)	2.32 ± 0.54[*]	4.24 ± 0.75	2.63 ± 0.62[*]
Cardiac risk ratio (LDL/HDL-C)	0.62 ± 0.08[*]	2.14 ± 0.67	1.12 ± 0.27[*#]

Values are represented as mean ± SEM coenzyme Q10; [*] Significant change in comparison with diabetic without treatment at P < 0.05; [#] Significant change in comparison with control at P < 0.05; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol
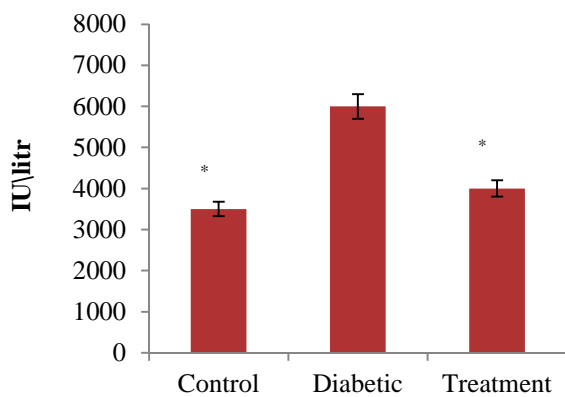
Figure 1. The effect of coenzyme Q10 on serum alkaline phosphatase activity in alloxan-induced diabetic rats
*Significant change in comparison with diabetic without treatment at $P < 0.05$
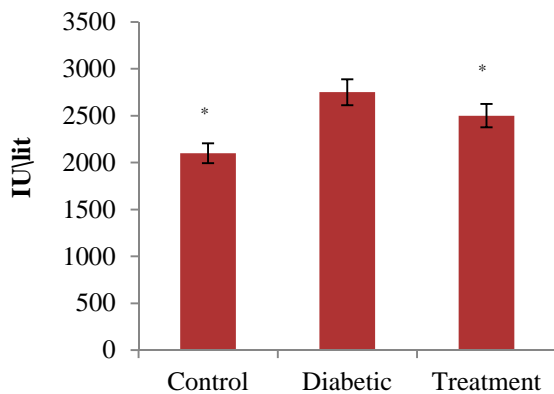
Figure 2. The effect of coenzyme Q10 on seruml alanine aminotransferase activity in alloxan-induced diabetic rats
*Significant change in comparison with diabetic without treatment at $P < 0.05$
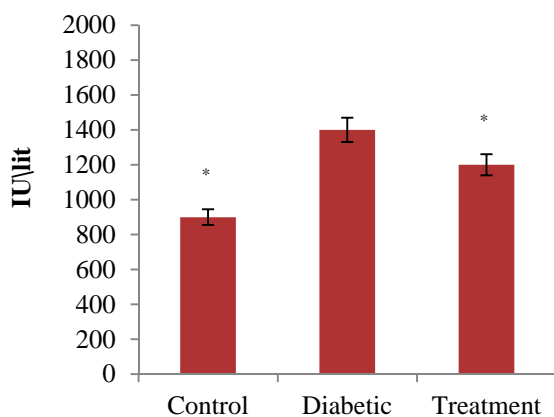
Figure 3. The effect of coenzyme Q10 on serum aspartate aminotransferase activity in alloxan-induced diabetic rats
*Significant change in comparison with diabetic without treatment at $P < 0.05$

Discussion

Effect of coenzyme Q10 on serum lipid profile and atherogenic index

Diabetes significantly increased serum FBG, TG, TC, VLDL, and LDL concentrations in comparison with the control group. Treatment of diabetic animals with coenzyme Q10 significantly inhibited inc rease of serum FBG, TG, TC, VLDL, and LDL concentrations, atherogenic index, atherogenic coefficient, and cardiac risk ratio in comparison with the untreated diabetic animals. Furthermore, treatment of diabetic animals with coenzyme Q10 significantly inhibited decrease of serum HDL concentrations in comparison with the untreated diabetic animals. There are reports that natural antioxidant such as lycopene and natural phenolic compounds have hypolipidemic effects.[25,26] Furthermore, there are reports that coenzyme Q10 have hypolipidemic effects. Cicero et al. showed coenzyme Q10 could reduce serum lipoprotein (a) level in patients with high serum triglyceride levels.[27] Moreover, Gao et al. showed coenzyme Q10 could reduce serum lipoprotein (a) level in patients with coronary artery diseases.[28] Also, Shojaei et al. showed coenzyme Q10 could reduce serum levels of lipoprotein (a) and lipids in Maintenance Hemodialysis Patients on Statin Therapy.[29]

Results of our study are in accordance with others researchers' study that showed coenzyme Q10 could reduce serum lipid and lipoprotein level. Therefore, natural antioxidant with hypolipidemic effects could prevent or be helpful in reducing the complications of lipid profile seen in diabetes patients. The mechanisms by which coenzyme Q10 can decrease high serum lipid level is not well known. The mechanism of hypolipedemic and antiatherogenic action of natural antioxidant may be due to the inhibition of dietary lipid absorption in the intestine or its production by liver or stimulation of the biliary secretion of cholesterol and cholesterol excretion in the faces.[30,31] Moreover, coenzyme Q10 is a lipid-solu ble molecule, and it is present in sufficient amounts in lipoprotein (a). Supplementation with coenzyme Q10 can inhibit expression of lipoprotein (a) receptor and result in decreased serum lipoprotein (a).[32] Also, the mechanism of hypolipedemic and antiatherogenic action of natural antioxidant may be due to the inhibition of glycation lipoproteins, enzymes and proteins that involve lipid and lipoprotein metabolism.[33]

Effect of coenzyme Q10 on serum ALP, ALT, and AST activity

Serum ALP, ALT, and AST activity as markers of liver function significantly were increased in the untreated diabetic animals in comparison with the control group. Treatment of the diabetic animals with coenzyme Q10 could significantly inhibit increase of serum ALP, ALT, and AST activity in comparison with the untreated diabetic animals. Treatment by coenzyme Q10 could maintain serum ALP, ALT, and AST activity of the treated animal at the same level as that of the control group. ALP, ALT, and AST are considered to be biochemical markers for assessing liver function. Hepatotoxicity is evidenced by an elevation of the serum marker enzymes.[34,35]

There are reports that natural antioxidant such as leptin and melatonin reduced these liver enzymes markers.[36,37] Also, there are reports that coenzyme Q10 have hepatoprotective effects.[38] Mabuchi et al. showed coenzyme Q10 could reduce serum ALT and AST activities.[38] Moreover, Ali et al. showed coenzyme Q10 could reduce serum ALT and AST activities on CCl_4-induced liver injury in rats.[39] Also, Amimoto et al. that is chemically damaged livers pretreated with coenzyme Q10 showed a decrease in the activity of serum ALT and AST.[40] Results of our study are in accordance with others researchers' study that showed coenzyme Q10 could reduce serum ALT, AST, and ALP activities. Therefore, natural antioxidant with hepatoprotective action could prevent or be helpful in reducing the complications of hepatic damage seen in diabetes patients.[41]

Researchers indicated that coenzyme Q10 is found to possess a good antioxidant activity.[15] Also researchers reported the role of oxidative stress as a central factor in onset and progression of diabetic complications such as hyperlipemia and hepatic damage.[4,42] Therefore, numerous reports and our results that showed efficacy of antioxidative supplements administration in the prevention of diabetic complications. Since antioxidant therapy is as one of the most important treatment strategies for diabetic patients for the prevention and slowing of diabetic complications progression such as hyperlipemia and hepatic damage.

This study has limitation in which we assumed the groups 2 diabetic without treatment with placebo.

Conclusion

This study showed that coenzyme Q10 has beneficial effects, in reducing the elevated serum lipid profile, atherogenic index and liver enzyme markers of alloxan-induced-diabetic rats. Hence, attenuation of lipid profile, atherogenic index and liver enzyme markers can decrease the risk of cardiovascular death and hepatic damage in diabetic patients.

Acknowledgments

Conflict of Interests

Authors have no conflict of interests.

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Normal range of bleeding time in urban and rural areas of Borujerd, west of Iran

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Original Article

Abstract

BACKGROUND: Bleeding time (BT) is the oldest and simplest test for assessing the platelets (Plts) function. BT can affect by several factors such as race and diet, which has a wide reference range. The aim of this project is to determine the normal range of BT in Borujerd, Iran. Determining the normal range of BT can help us to modify the definition of bleeding disorder and aspirin resistance.

METHODS: This was cross-sectional study carried out in 2011-2012. Subjects with a history of coagulation disorders or a positive family history of coagulation disorders, consumption of anti-Plts, anti-histamines, and phenothiazine in the previous month and subject with Plt less than 150,000 were excluded. The samples were 505 volunteers who were referred from 16 urban and 9 rural clusters to research center. BT of the samples was determined according to Ivy simplate method considering national standard protocol in the selected persons. Normal range was calculated as mean ± 2 standard deviation.

RESULTS: Of 505 volunteers, 50.4% were female. The range of BT was 2.8-2.95 min with mean of 2.79 ± 0.78 min. Range and mean of BT in women was 2.83-3.06 min and 2.88 ± 0.87 min, and range and mean of BT in men was 2.7-2.9 min and 2.69 ± 0.67 min; this difference was significant (P = 0.012). BT in urban and rural participants was 2.78 ± 0.79 and 2.77 ± 0.73 min. There was no significant difference between BT in urban and rural participants.

CONCLUSION: The normal range of BT in Boroujerd was in the lower limit of the normal universal range. In this study, BT was significantly different in both genders, but its correlation with age, blood group, and place of residency was not significant.

Keywords: Bleeding Time, Blood Platelet, Iran

Introduction

Platelets (Plts) play an important role in blood clotting and hemostasis. The function of Plts is assessed by various methods. Bleeding time (BT) is the oldest test for assessing the Plts function. The benefits of this test are that it is quick and facile. BT is defined as the time from the moment that incision is made to the point where bleeding ceases. Several factors such as Plt count, hematocrit and temperature can influence BT.[1] Furthermore, it is reported that race and diet can affect the Plt aggregation.[2]

According to our knowledge, it is the first time that BT has been assessed in this amount of sample volume in Iran. The aim of this study was to evaluate BT in Borujerd population, a city in the west of Iran. Determining the normal range of BT can help us to modify the definition of bleeding disorder and aspirin resistance. Recently, aspirin resistance is presented as a predictor of cardiovascular disease.

Materials and Methods

This cross-sectional study was done in Borujerd, a city in the west of Iran in 2011-2012. The Research and Ethics Committee of Lorestan University of Medical Sciences, Iran, approved this study (No.

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1255). Written informed consent was taken from all participants. Due to lack of similar published information, primarily a pilot study was designed, and based on its measured variance, the sample size was determined. The pilot study was performed on 30 subjects. According to the results of the pilot study, a sample size of 580 persons, including 290 in each gender groups was estimated by a power of 0.95.

Inclusion criteria comprised of volunteers aged 35 years or more, and signature of written consent. The subjects with a history of coagulation disorders or a positive family history of coagulation disorders, consumption of anti-Plts (such as aspirin and indomethacin), anti-histamines, and phenothiazine in the previous month and the subjects with Plts less than 150,000 were excluded. The samples were consecutively selected from the patients who were referred to 16 urban and 9 rural health and treatment centers. A trained nurse recorded their medical history according to the questionnaire. BT of the samples was determined according to Ivy simplate method considering national standard protocol in the selected persons.

For performing the Ivy simplate method, a blood pressure cuff was placed on the upper arm and then was inflated to 40 mmHg. An incision with a length of 8 mm and a depth of 1 mm was made by a lancet in the anterior section of the underside of the forearm in an area without superficial veins. The beginning of incision until the time that bleeding stopped was described as BT.[3] Digital chronometers were used to measure time, and all samplers had similar chronometers. A standard filter paper was used every 30 s to draw off the blood until the blood stopped completely. The blotting paper was coded and was sent to a research center to fill table times according to codes of blotting paper of each sample and was reevaluated by a second observer. One sampler was trained for performing the test. Due to painfulness of this technique, the process was performed just once for each participant, but two observers recorded all the results: one person recorded the results during the test, and the other one interpreted the results and recorded them in the related forms. When there were differences in sample reading, we considered the mean of the two results.

Statistical analysis
The recorded data were analyzed by SPSS for Windows (SPSS 13.0, SPSS Production Facility, Chicago, IL, USA). The P value of < 0.05 and confidence interval of 95% were considered as statistically significant. Continuous parameters were described as mean ± standard deviation (SD). The data were analyzed with Student's t-test, and one-way ANOVA test categorical data were described as percentages and analyzed by chi-square test. Normal range was calculated as mean ± 2SD.

Results
The present study was performed on 505 volunteer subjects (75 subjects were excluded from the study). About 50.4% of the participants were female, and 49.60% were male. The youngest participant was 35 years old, and the oldest one was 88 years old. In this study, the normal range of BT in the participants was 1.23-4.35 min with a mean of 2.79 ± 0.78 min. The normal range and mean of BT in women were 1.14-4.62 min and 2.88 ± 0.87 min, and the normal range and mean of BT in men were 1.35-4.03 min and 2.69 ± 0.67 min, respectively. Independent t-test showed a significant difference between BT in women and men (t = 2.520, P = 0.012).

The BT according to the blood group and Rh are shown in table 1. The difference between BT in blood groups as was not significant (P = 0.590). The BT according to the age groups is shown in table 2. There was no significant difference between BT in different age groups by one-way ANOVA test (P = 0.683).

Three hundred and sixty-three participants (72%) had been living in urban areas. BT in urban and rural participants was 2.78 ± 0.79 and 2.77 ± 0.73 min, respectively. There was no significant difference between BT in urban and rural participants.

Table 1. The bleeding time according to the blood groups and Rh represented by mean ± standard deviation

	Total	A (n = 152)	B (n = 122)	AB (n = 35)	O (n = 196)	P[*]
Total	2.56 ± 1.27	2.79 ± 0.77	2.80 ± 0.80	2.64 ± 0.59	2.79 ± 0.89	0.59
Rh positive (n = 468)	2.57 ± 1.27	2.75 ± 0.78	2.84 ± 0.86	2.64 ± 0.59	2.73 ± 0.72	0.66
Rh negative (n = 37)	2.50 ± 1.27	3.17 ± 0.60	2.42 ± 0.45	-	3.31 ± 1.02	0.37[*]
P[**]	0.42	0.49	0.51	-	0.53	-

[*] One-way ANOVA test; [**] Student's t-test

Table 2. The bleeding time according to the age groups

Age (year)	n (%)	Mean ± SD (min)	Normal range (min)
35-44	116 (23.1)	2.86 ± 0.806	1.248-4.472
45-54	151 (30.0)	2.84 ± 0.823	1.194-4.486
55-64	106 (21.1)	2.79 ± 0.825	1.140-4.440
≥ 65	130 (25.8)	2.63 ± 0.635	1.360-3.900

SD: Standard deviation; P = 0.683 (one-way ANOVA test)

Discussion

BT is the oldest test for assessing the Plts function. This test is a quick and facile and inexpensive test. In this study, the normal range of BT in the participants was 1.23-4.35 min with a mean of 2.79 ± 0.78 min. Although, the normal range of BT is generally defined as 2-10 minutes. BT in women was more prolonged than in men. The difference between BT in blood groups was not significant. There was no significant difference between BT in different age groups. According to our knowledge, it is the first time that BT has been assessed in this amount of sample volume in Iran.

In this study, the normal range of BT in the participants was 1.23-4.35 min with a mean of 2.79 ± 0.78 min. Although, the normal range of BT is generally defined as 2-10 min. However, it is defined as < 7.1 min[4] and 1-9 min[5,6] in other references. BT in our study is in the normal reported ranges, but it is in the lower limit of reported ranges. This may due to the differences between the properties of Borujerd population and the world population. For example, Kickler reported that race and diet can affect the Plt aggregation.[2] Knowing the normal range of BT is important because reported universal ranges may misguide the physicians in dose adjustment of anti-Plts.

In our study, BT in women was more prolonged than in men. This finding is in accord with the study by Valeri et al.[7] They assessed BT in 44 healthy male and female volunteers. They reported that, at +32° C, BT in women had been longer than in men. Also, Uden et al. evaluated BT in 195 cases with scoliosis and in 318 controls.[8] They reported that BT in women had been longer than in men. Furthermore, Chen et al. stated that BT had been longer in females (26 participants) than in males (25 participants) (11.4 ± 0.9 vs. 8.3 ± 0.7).[9] Also, Roy et al. declared that in 261 medical students who participated in their study in Nepal, BT had been longer in women than in men.[10]

In our study, the difference between BT in blood groups was not significant. This finding is not in accord with the study by Caekebeke-Peerlinck et al.[11] They evaluated BT in healthy volunteers and reported that BT had been longer in individuals with blood group O than in individuals with non-O blood groups. Also, Adhikari et al. stated that BT in individuals with blood group O had been longer than in other blood groups.[12] In contrast, Mahapatra and Mishra reported that BT in blood group AB had been longer than in other blood groups.[13]

In our study, there was no significant difference between BT in different age groups. It is in contrast with Reilly and FitzGerald's study. They reported that BT had been briefer in the older applicants.[14] Also, our finding is not in accord with Jorgensen et al.'s study. They stated that the BT in men had been shortening in older participants.[15]

In our study, there was no meaningful correlation between BT and Plt count more than 150,000. Ramanathan et al. assessed the correlation between Plt count and BT in patients with preeclampsia.[16] They reported that only when Plt count was lower than 100,000/mm³, BT had been correlated with Plt count. Harker and Slichter evaluated the relationship between of BT and Plt count in the patients with thrombocytopenia with the Plt count between 10,000 and 100,000/µl. They reported that there was an inverse relationship between BT and Plt count in them.[17] None of these studies reported a correlation between Plt count more than 150,000 and BT.

Conclusion

Our study showed that the normal range of BT in Borujerd was different from normal universal ranges. Also, in this study BT was significantly different in two genders, but its correlation with age, blood group, and place of residency was not significant.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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Dietary phytochemical index and subsequent changes of lipid profile: A 3-year follow-up in Tehran Lipid and Glucose Study in Iran

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Original Article

Abstract

BACKGROUND: High intakes of phytochemical-rich foods have beneficial effects on lipid profiles and cardiovascular disease (CVD). In this study, we assessed the association between the dietary phytochemical index (PI) and changes in lipid profile after 3-year follow-up among Iranian adults.

METHODS: This longitudinal study was conducted in 1983 subjects, aged 19-70 years, selected among participants of the Tehran Lipid and Glucose Study in Iran. Dietary data were collected by using a validated semi-quantitative food frequency questionnaire with 168 food items at baseline. PI was calculated based on daily energy derived from [(phytochemical-rich foods kcal/total daily energy intake kcal) × 100]. Lipid profile was measured at baseline and after 3 years and changes in serum lipid profiles were assessed during 3-year follow-up.

RESULTS: The mean age of participants was 40.4 ± 13.0 years; participants in the highest PI quartile category were more likely to be older. After 3 years of follow-up, total cholesterol was significantly lower in the highest quartile compared with lower quartile of PI in men (181 ± 3 vs. 189 ± 3, *P* for trend < 0.05). There were significant inverse association between dietary PI and 3 years changes of total cholesterol [β = −5.6, 95% confidence interval (CI) = −9.3, −1.8], triglycerides (β = −13.7, 95% CI = −24.6, −2.8), and non-high-density lipoprotein cholesterol (HDL-C) (β = −6.2, 95% CI = −10.8, −1.5), in highest quartile of PI in men. Lipid profiles showed no significant changes over the study period in women.

CONCLUSION: Higher dietary PI is associated with 3 years improvement of total cholesterol, triglycerides, and non-HDL-C. Higher consumption of phytochemical-rich foods is recommended to prevent CVD.

Keywords: Phytochemical, Triglyceride, Cholesterol, Fruit and vegetables, Whole Grains

Introduction

Cardiovascular disease (CVD) is one of the major public health problems that lead to disability and mortality.[1] Hypercholesterolemia has been investigated as a major risk factor for CVD.[2] Worldwide prevalence of hypercholesterolemia was estimated 39% (37% in men and 40% in women) in 2008; it was associated to 2.6 million deaths and 29.7 million disability-adjusted life years.[1] In Iran, prevalence of hypercholesterolemia was reported 51.7% (48.8% in men and 54.7% in women) in 2008.[3] Prospective studies, have also shown that increased triglycerides and decreased high-density lipoprotein cholesterol (HDL-C) levels are associated with CVD independent of traditional risk factors, suggesting that improvement of these abnormalities as secondary therapeutic targets have protective effects.[4,5] Moreover, non-HDL-C that comprises all atherogenic apolipoprotein B (Apo B) is a better measure for evaluation atherogenic particles and prediction of cardiovascular events than low-density lipoprotein cholesterol (LDL-C).[6]

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It has been demonstrated that dietary modification is related to lipid profile promotion, and hence to CVD prevention.[7-10]

The previous studies have shown that a vegetarian diet compared with an omnivorous one has an inverse association with lipid profiles.[11,12] Vegetarian diet provide higher amount of fruits, vegetables, whole grains, nuts and soy, all of which are associated with lower risk of CVD through lipid profile reduction.[13-15] Phytochemicals such as phytosterols and phenolic compounds are bioactive compounds that are found abundantly in plant foods and protect against cardiovascular events by reducing prothrombic and inflammatory status and improving endothelial function.[16] It has been suggested that phytochemicals have an important role in lipid metabolism that causing decrease in lipid profile.[17] Regarding health-promotional properties of phytochemicals, for the first time, McCarty proposed a "phytochemical index" (PI), defined as the percent of dietary calories derived from foods rich in phytochemicals, and suggested that PI could be used as an index of total dietary phytochemical content.[18] This index is a simple method for assessment of phytochemical intake that, despite its limitations, could provide important background for diet quality and may have high practical and clinical uses.[19] Previous clinical trials have documented beneficial effect of phytochemical supplements on lipid profile.[20-23] Recently, in a cross-sectional study, we have indicated that subjects in higher quartile of dietary PI intake have lower risk of hypertriglyceridemia [0.36, 95% confidence interval (CI) = 0.47-0.86].[24] However, to our knowledge, no prospective population-based studies of PI and lipid profile have been published. Therefore, in this population-based longitudinal study, we assessed the baseline dietary PI in relation to 3 years changes of lipid and lipoprotein levels among Tehranian adults.

Materials and Methods

Study design and subjects
This study was conducted within the framework of the Tehran Lipid and Glucose Study (TLGS) in Iran.[25] Briefly, TLGS, a community-based prospective study that began in 1999 and data collection is ongoing at 3-year intervals, is being conducted to investigate and prevent non-communicable diseases (NCDs) by promoting healthy lifestyles and reducing NCD risk factors in a representative sample of residents, aged ≥ 3 years, from district 13 of Tehran, Iran. Baseline examination of the current study was included 2799 adults aged 19-70 years with complete data (demographic, anthropometric, biochemical, and dietary data), participated in the third phase of TLGS (2006-2008). Participants were excluded from the final analysis if they reported implausible energy intake (< 800 kcal/d or ≥ 4200 kcal/d), were on specific diets (n = 232), or had no follow-up information on anthropometrics and biochemical measurements at the second examination (2009-2011) (n = 629); finally 1938 participants (845 men and 1093 women) were included in the analysis. The mean duration of the follow-up was approximately 3 years.

Informed written consents were obtained from all participants and the study protocol was approved by the research council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences.

Dietary assessment and PI calculation
Dietary data were collected by using a validated semi-quantitative food frequency questionnaire (FFQ) with 168 food items[26] at baseline. Trained dietitians with at least 5 years of experience in the TLGS survey interviewed participants, face to face, and asked them about their consumption frequency for each food item consumed during the past year on a daily, weekly, or monthly basis. The validity of the FFQ was previously evaluated by comparing food groups and nutrient values determined from the questionnaire with values estimated from the average of twelve 24-h dietary recall surveys.[26,27] Portion sizes of consumed foods that were reported in household measures were then converted to grams. The United States Department of Agriculture (USDA) food composition table (FCT) was used to calculated energy and nutrient intakes. The Iranian food composition table was also used for some national foods that are not listed in the USDA FCT.[28,29]

PI was calculated based on the McCarty equation:[18]

$$PI = \frac{\text{dietary energy derived from phytochemical on ported in house}}{\text{total daily energy intake (kcal)}} \times 100$$

Fruits and natural fruit juices, vegetables and natural vegetables juices, whole grains, legumes, nuts, seeds, olives, and olive oil were defined as phytochemical-rich foods.

Lifestyle assessment
Lifestyle data, including physical activity level, smoking status, and educational level were collected at baseline. Physical activity level was assessed using

the Krishka et al. questionnaire.[30] The frequency and time spent on light, moderate, hard and very hard intensity activities, according to the list of common activities of daily life, over the past year were obtained. Physical activity levels were expressed as metabolic equivalent hours per week (METs h/week). Subjects who had smoked daily or occasionally were considered to be current smokers and those who had never smoked or those who given up smoking as non-smokers.

Laboratory measurements

Blood samples were taken after 12-14 h overnight fasting at baseline and after 3 years. Total cholesterol level was measured using enzymatic colorimetric analysis with cholesterol esterase/cholesterol oxidase. Triglyceride level was measured using by enzymatic colorimetric analysis with glycerol phosphate oxidase. HDL-C was measured after precipitation of the Apo B containing lipoproteins with phosphotungstic acid. LDL-C was calculated according to the Friedwald equation if triglyceride concentration was less than 400 mg/dl. Analyses were performed using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran) and a selectra 2 auto-analyzer (Vital Scientific, Spankeren, The Netherlands). Inter- and intra-assay coefficients of variation of all assays were all < 5%.

Statistical analysis

Dietary PI at baseline was divided into quartiles; participant characteristics, and baseline and 3 years changes of lipid and lipoprotein levels, were compared across quartile categories of PI, using the general linear models adjusted for age or the chi-square test. The mean dietary intakes of participants were compared across quartile categories of PI using general linear model with adjustment for age and energy intake. To assess the overall trends of the lipid and lipoprotein mean across PI quartiles, the median of PI in each quartile was used as a continuous variable in the logistic regression models. The mean of 3 years changes in lipid and lipoprotein levels were calculated as [(second levels−baseline levels)/baseline levels]. Multiple regression models were used to evaluate the association between dietary PI and changes in serum total cholesterol, triglycerides, LDL-C and HDL-C. Subjects in the first PI quartile were considered as the reference group. To determine the association between each phytochemical-rich food groups with 3 years changes in lipid profile, we also categorized energy adjusted intakes of whole grains, vegetables, fruits, legumes, nuts, soy, olives and olive oil, into quartiles. Mean change of each lipid profile measure associated with each category of dietary PI or phytochemical-rich food, compared with the reference group and their 95% CIs were estimated by using the multiple regression models with adjustment for potential confounder variables. The variables adjusted in the models were sex, age at baseline (years, continuous), body mass index (kg/m², continuous), education (four categories), smoking (yes or no), physical activity (MET-h/week, continuous), total energy intake (kcal/d), dietary carbohydrate (% of energy), fat (% of energy), and protein (% of energy). A linear trend test was performed by considering each ordinal score variable as a continuous variable in the model. All statistical analysis were conducted using the SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA), with *P*-values < 0.05 was considered as significant.

Results

The mean age of participants at baseline was 41.4 ± 13.5 and 39.6 ± 12.6 years in men and women, respectively; about 53% of participants were women. The mean 3-year changes were: serum cholesterol 1.1 ± 1.0 mg/dl in men and 1.9 ± 0.9 mg/dl in women; triglycerides −5.8 ± 2.8 and −4.9 ± 1.7 mg/dl; HDL-C 4.1 ± 0.2 mg/dl and 5.9 ± 0.2 mg/dl and LDL-C −1.9 ± 0.9 and −3.0 ± 0.8 mg/dl in men and women, respectively.

The mean PI was 29.8 ± 12.3; 28.5 ± 12.1 in men, and 30.9 ± 12.3 in women. The dietary PI ranged from 19.6 to 35.5 in men and 21.8 to 37.9 in women (Table 1). Participants in the highest PI quartile category were more likely to be older compared with the lowest PI quartile (35 vs. 48 years in men and 36 vs. 45 in women, P for trend < 0.001).

Lipid and lipoprotein levels of participants by categories of dietary PI at baseline and after 3 years of follow-up are presented in table 2. After 3 years of follow-up, total cholesterol levels were significantly lower in the highest compared with the lowest PI quartile category in men (181 ± 3 vs. 189 ± 3 mg/dl, *P* for trend < 0.05); moreover, 3 years change of total cholesterol was inversely associated with dietary PI (3.9 ± 2.1 mg/dl decrease in the highest PI quartile vs. 4.3 ± 2.1 mg/dl increase in the lowest PI quartile, P for trend < 0.05). At baseline and after 3-year level of triglyceride decreased across quartile of PI in men and women, but were not significant. HDL-C level had not significant changes across quartile of PI in men and women at baseline and after 3 years. LDL-C level decreased across quartile of PI in men and

women at baseline and after 3 years, but it was not constant. The levels of triglyceride, HDL-C, and LDL-C had not significant changes across the PI quartile during the 3-year follow-up.

Table 1. Demographic characteristics of participants by categories of dietary phytochemical index: Tehran Lipid and Glucose Study

Demographic characteristics	Dietary phytochemical index							
	Men				Women			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Dietary phytochemical index								
Range	< 19.6	19.6-27.0	27.1-35.5	> 35.5	< 21.8	21.8-29.6	29.7-37.9	> 37.9
Mean	16.5 ± 0.5	24.7 ± 0.5	31.4 ± 0.5	41.1 ± 0.5	19.1 ± 0.4	26.8 ± 0.4	34.5 ± 0.4	43.2 ± 0.4
Age 2006-2008 (year)	35.1 ± 0.8	39.2 ± 0.8	42.9 ± 0.8	$48.4 \pm 0.8^{*}$	36.6 ± 0.7	37.2 ± 0.7	39.3 ± 0.7	$45.2 \pm 0.7^{*}$
Physical activity (MET-h/week)								
Job activity	38.9 ± 4.7	27.1 ± 4.5	31.4 ± 4.5	23.1 ± 4.7	23.2 ± 2.2	24.6 ± 2.2	23.4 ± 2.2	20.6 ± 2.3
Leisure time activity	10.4 ± 1.3	13.4 ± 1.3	9.5 ± 1.3	13.0 ± 1.3	8.5 ± 0.8	8.2 ± 0.7	10.0 ± 0.7	9.0 ± 0.8
Total	49.4 ± 4.9	40.5 ± 4.7	41.0 ± 4.7	36.1 ± 4.9	31.7 ± 2.4	32.8 ± 2.4	33.4 ± 2.4	29.6 ± 2.4
Current smoker (%)	24.9	24.5	24.2	18.5	1.8	2.2	0.4	3.3
Education status (%)								
Illiterate	1.4	0.9	1.0	1.4	2.2	2.6	3.3	4.4
Primary education	10.0	7.1	0.0	11.1	0.0	10.7	0.0	0.0
Academic education	80.0	85.8	78.6	66.7	92.0	82.2	95.0	100.0
Advanced academic education	10.0	7.1	21.4	22.2	8.0	7.1	5.0	0.0

Mean $\pm$ SEM; * P < 0.05 (chi-square test or age-adjusted general linear models were used); SEM: Standard error of mean

Table 2. Lipid profile of participants by categories of dietary phytochemical index at baseline and after 3 years of follow-up: Tehran Lipid and Glucose Study

Lipid profile	Dietary phytochemical index							
	Men				Women			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Triglycerides (mg/dl)								
Baseline	161 ± 7	167 ± 7	158 ± 7	155 ± 7	132 ± 4	126 ± 4	127 ± 4	130 ± 4
After 3-years	158 ± 7	166 ± 7	150 ± 7	144 ± 7	127 ± 4	122 ± 4	124 ± 4	124 ± 4
Changes	-2.7 ± 5.9	-1.5 ± 5.7	-8.1 ± 5.7	-10.8 ± 5.9	-5.4 ± 3.4	-3.9 ± 3.4	-4.7 ± 3.4	-5.7 ± 3.5
Total cholesterol (mg/dl)								
Baseline	185 ± 3	190 ± 3	183 ± 3	185 ± 3	186 ± 2	187 ± 2	189 ± 2	184 ± 2
After 3-years	189 ± 3	192 ± 3	185 ± 3	$181 \pm 3^{*}$	189 ± 2	188 ± 2	190 ± 2	185 ± 2
Changes	4.3 ± 2.1	2.3 ± 2.0	1.6 ± 2.0	$-3.9 \pm 2.1^{*}$	3.5 ± 1.8	1.2 ± 1.8	1.7 ± 1.8	1.2 ± 1.9
HDL-C (mg/dl)								
Baseline	38.0 ± 0.6	38.1 ± 0.5	38.1 ± 0.5	38.4 ± 0.6	45.6 ± 0.6	45.7 ± 0.6	45.5 ± 0.6	44.8 ± 0.6
After 3-years	42.0 ± 0.6	42.8 ± 0.6	41.8 ± 0.6	42.3 ± 0.6	51.0 ± 0.7	51.8 ± 0.6	51.6 ± 0.6	51.0 ± 0.7
Changes	4.0 ± 0.4	4.6 ± 0.4	3.7 ± 0.4	3.9 ± 0.4	5.4 ± 0.5	6.1 ± 0.5	6.0 ± 0.5	6.1 ± 0.5
LDL-C (mg/dl)								
Baseline	116 ± 2	120 ± 2	113 ± 2	115 ± 2	115 ± 2	116 ± 2	117 ± 2	113 ± 2
After 3-years	116 ± 2	117 ± 2	113 ± 2	110 ± 2	113 ± 2	112 ± 2	114 ± 2	109 ± 2
Changes	0.9 ± 1.8	-2.7 ± 1.8	-0.2 ± 1.8	-5.5 ± 1.8	-1.2 ± 1.6	-4.1 ± 1.6	-3.4 ± 1.6	-3.4 ± 1.6

Mean $\pm$ SEM; * P < 0.05 (age-adjusted general linear models were used); SEM: Standard error of mean; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

The mean dietary intake of men and women across dietary PI quartile categories are presented in table 3. Dietary energy and fat intake decreased significantly across quartiles of PI (P for trend < 0.001), while dietary intake of carbohydrate, protein, and vitamin C significantly increased (P for trend < 0.001) in both men and women. Dietary intakes of whole grains, fruits, vegetables, seeds, nuts, and olive oil in the highest quartile category of PI, were significantly higher than the lowest quartile categories in men and women.

The associations between baseline PI with 3 years changes in total cholesterol, triglycerides, LDL-C, and HDL-C adult participants are shown in table 4. After adjustment for potential confounding variables, there was significant inverse association between highest quartile category of dietary PI with changes in triglycerides, total cholesterol, HDL-C, and non-HDL-C in men (P for trend < 0.01), while dietary PI had no significant association with changes in lipid profile across quartile categories in women.

Discussion

In this first longitudinal study of PI and lipid profile, we found that increased energy intakes from phytochemical rich foods, presented as dietary PI, could have favorable effects on subsequent changes of triglycerides, total cholesterol, and non-HDL-C levels in men. In addition, during the follow-up, we found a significant reduction in the total cholesterol levels in men who had higher phytochemicals index. No significant association was observed between dietary PI and 3 years changes of lipid and lipoprotein levels.

It is well-known that non-pharmacological agents such as bioactive food components and functional foods can improve lipid profile.[31] Epidemiologic evidence indicate that the incidence of cardiovascular disease is lower in populations who consume phytochemical-rich diet; this effects are mainly attributed to functional properties of phytochemicals including improvement of lipid profile, anti-inflammatory, anti-prothrombotic and anti-oxidative properties.[32-36] The Mediterranean diet is the best example of a phytochemical-rich diet; recently, two studies have reported that Mediterranean and phytochemicals-rich diets reduce total cholesterol, LDL-C and non-HDL-C levels, but their decrease was greater in phytochemicals-rich diet than in

the Mediterranean diet. Besides, HDL-C level has increased only in phytochemicals-rich diet.[10,37] Lukaczer et al.[38] have demonstrated that phytochemicals-rich diet is more effective than American Heart Association diets to manage lipid profiles. These findings suggest that phytochemicals may have further effect on lipid profile improvement.

It has suggested that phytosterols are responsible for phytochemical-related lipid reduction. Phytosterols are a subclass of phytochemicals with potent lipid lowering properties; several studies have evaluated cholesterol-lowering effect of phytosterols.[39,40] Studies showed that enrichment of food products with phytosterols could effectively improve lipid and lipoprotein levels.[41,42] Main mechanism by which phytosterols reduce cholesterol by competing with cholesterol for micellar incorporation, hence inhibiting its intestinal uptake, however the reason of LDL-C reduction is unknown.[43] It seems, frequency of phytosterols intake, also, affects cholesterol level as multiple-consumption of these has a greater effect compared with single-consumption.[39]

Moreover, some phytochemicals bind to peroxisome proliferator-activated receptors which regulate lipid metabolism, promote uptake, utilization, and catabolism of fatty acids by up-regulation of genes involved in fatty acid transport and peroxisomal and mitochondrial fatty acid β-oxidation.[17] In addition, animal studies have shown that phytosterols up-regulate hepatic ABCG5 transporters and result in cholesterol reduction.[44]

Conflict of Interests

Authors have no conflict of interests.

Table 3. Mean dietary intakes of participants by categories of dietary phytochemical index: Tehran Lipid and Glucose Study

Dietary intake	Dietary phytochemical index							
	Men				Women			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Energy intake (kcal/d)	2869 ± 41	2513 ± 40	2242 ± 40	1950 ± 41[*]	2618 ± 32	2252 ± 32	2131.0 ± 32.0	1724 ± 32[*]
Carbohydrate (% of total energy)	56.3 ± 0.4	57.9 ± 0.4	60.2 ± 0.4	61.9 ± 0.4[*]	52.9 ± 0.4	55.3 ± 0.4	57.1 ± 0.4	60.8 ± 0.4[*]
Fat (% of total energy)	31.6 ± 0.4	30.4 ± 0.4	28.9 ± 0.4	27.5 ± 0.4[*]	35.2 ± 0.4	33.4 ± 0.4	32.3 ± 0.4	29.3 ± 0.4[*]
Saturated fat (g/d)	29.9 ± 1.3	26.9 ± 1.3	26.4 ± 1.3	23.6 ± 1.4[*]	28.7 ± 0.5	27.2 ± 0.5	26.8 ± 0.5	23.0 ± 0.5[*]
Monounsaturated fat (g/d)	28.1 ± 0.5	28.1 ± 0.5	27.0 ± 0.5	25.2 ± 0.5[*]	29.5 ± 0.5	28.8 ± 0.5	28.2 ± 0.5	24.6 ± 0.5[*]
Polyunsaturated fat (g/d)	17.3 ± 0.4	17.3 ± 0.4	16.1 ± 0.4	15.2 ± 0.4[*]	18.3 ± 0.4	17.8 ± 0.4	16.9 ± 0.4	14.6 ± 0.4[*]
Protein (% of total energy)	12.9 ± 0.1	13.6 ± 0.1	13.6 ± 0.1	14.4 ± 0.1[*]	12.9 ± 0.1	13.5 ± 0.1	13.7 ± 0.1	14.3 ± 0.1[*]
Total fiber (g/d)	39.0 ± 1.4	40.1 ± 1.3	40.1 ± 1.3	43.4 ± 1.4	32.2 ± 0.9	35.4 ± 0.8	37.6 ± 0.8	38.9 ± 0.9[*]
Total carotenoids (μg/d)	6524 ± 385	8986 ± 349	9980 ± 350	11225 ± 384[*]	6919 ± 416	9573.0 ± 383	12457 ± 382	13588 ± 426[*]
Vitamin E (mg/d)	10.7 ± 0.3	11.6 ± 0.3	11.6 ± 0.3	11.6 ± 0.3	11.7 ± 0.3	11.9 ± 0.3	12.1 ± 0.3	11.9 ± 0.3
Vitamin C (mg/d)	77.2 ± 5.5	135.7 ± 5.0	164.0 ± 5.0	177.8 ± 5.5[*]	89.3 ± 5.0	144.1 ± 4.6	175.0 ± 4.6	201.8 ± 5.1[*]
Whole grains (g/d)	10.2 ± 6.4	75.1 ± 5.8	134.9 ± 5.8	208.3 ± 6.4[*]	8.7 ± 5.1	60.0 ± 4.7	110.0 ± 4.6	154.3 ± 5.2[*]
Fruits (g/d)	142 ± 17	343 ± 16	450 ± 16	502 ± 17[*]	178 ± 15	378.0 ± 14	430 ± 14	559 ± 15[*]
Vegetables (g/d)	202 ± 11	258 ± 10	284 ± 10	311 ± 11[*]	238 ± 13	288.0 ± 12	374 ± 12	384 ± 13[*]
Legumes (g/d)	13.3 ± 1.8	16.9 ± 1.6	17.6 ± 1.6	17.8 ± 1.8[*]	10.7 ± 1.1	14.5 ± 1.0	17.3 ± 1.0	17.3 ± 1.2[*]
Seeds (g/d)	0.4 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	3.4 ± 0.4[*]	0.3 ± 0.3	1.7 ± 0.3	1.5 ± 0.3	3.0 ± 0.3[*]
Nuts (g/d)	3.7 ± 0.7	6.2 ± 0.7	10.2 ± 0.7	11.0 ± 0.7[*]	3.0 ± 0.5	6.9 ± 0.5	7.4 ± 0.5	9.5 ± 0.5[*]
Olive oil (g/d)	0.2 ± 0.1	0.5 ± 0.09	0.7 ± 0.09	0.9 ± 0.1[*]	0.1 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	1.6 ± 0.2[*]
Soy sources (g/d)	0.6 ± 0.3	1.8 ± 0.2	1.3 ± 0.3	2.2 ± 0.3[*]	1.3 ± 0.4	1.9 ± 0.3	1.5 ± 0.3	2.8 ± 0.4

Mean ± SEM; [*] P < 0.05 (age- and energy-adjusted models were used); SEM: Standard error of mean

Table 4. The association of dietary phytochemical index with 3 years changes in lipid profiles in Iranian adults: Tehran Lipid and Glucose Study[*]

3-year lipid profile changes	Dietary phytochemical index					
	Men			Women		
	Q2	Q3	Q4	Q2	Q3	Q4
Triglycerides	−6.0 (−15.1, 2.9)	−11.4 (−21.1, −1.8)	−13.7 (−24.6, −2.8)[**]	−0.8 (−7.8, 6.1)	−0.2 (−7.5, 7.1)	−4.3 (−12.9, 4.3)
Total cholesterol	−1.6 (−4.7, 1.5)	−2.5 (−5.9, 0.8)	−5.6 (−9.3, −1.8)[**]	−1.6 (−4.4, 1.2)	−0.8 (−3.6, 2.2)	−1.7 (−5.1, 1.73)
HDL-C	0.7 (−2.6, 4.1)	−3.1 (−6.6, 0.5)	−3.6 (−7.7, 0.4)	0.8 (−2.9, 4.4)	0.4 (−3.4, 4.2)	−0.2 (−4.8, 4.3)
LDL-C	−2.7 (−7.5, 2.0)	−0.6 (−5.7, 4.5)	−4.9 (−10.7, 0.8)	−2.5 (−6.6, 1.6)	−0.9 (−5.2, 3.5)	0.7 (−4.5, 5.8)
Non-HDL-C	−2.5 (−6.3, 1.4)	−2.8 (−6.9, 1.3)	−6.2 (−10.8, −1.5)[**]	−2.5 (−6.1, 1.1)	−1.1 (−4.8, 2.6)	−1.9 (−6.4, 2.5)
Total cholesterol/HDL-C	−2.2 (−5.7, 1.4)	−0.2 (−4.1, 3.6)	−2.3 (−6.7, 1.9)	−1.7 (−5.0, 1.6)	−1.5 (−4.9, 2.0)	−1.2 (−5.3, 2.9)
LDL-C/HDL-C	3.6 (−8.6, 1.4)	1.5 (−3.8, 6.8)	−2.2 (−8.2, 3.7)	−2.3 (−7.1, 2.4)	−1.9 (−7.0, 3.1)	1.0 (−4.9, 7.0)
Triglyceride/HDL-C	−6.4 (−16.3, 3.6)	−9.1 (−19.7, 1.6)	−10.7 (−22.7, 1.3)	−1.2 (−9.1, 6.7)	−2.0 (−10.3, 6.2)	−4.1 (−13.9, 5.7)

Q1 was considered as reference group; [*] Data are β regression and 95% confidence interval [linear regression models were used with adjustment for age, total energy intake (kcal/d), dietary carbohydrate (% of energy), fat (% of energy), protein (% of energy), saturated fatty acid (kcal/d), mono-saturated fatty acid (kcal/d) and poly-saturated fatty acid (kcal/d)]; [**] P for trend < 0.05; Medians of dietary phytochemical index quartiles in men were 16.3, 24.4, 30.7, and 35.5 in the first, second, third, and fourth quartile categories, respectively and in women were 18.9, 25.3, 33.2, and 37.9 in the first, second, third, and fourth quartile categories, respectively; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

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In-hospital outcomes after primary percutaneous coronary intervention according to left ventricular ejection fraction

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Original Article

Abstract

BACKGROUND: The primary objective of primary percutaneous coronary intervention (pPCI) in patients with acute ST-segment elevation myocardial infarction (STEMI) is not only to restore the blood flow in the infarct-related artery, but also to save the patients' quality and duration of their life. Since left ventricular ejection fraction (LVEF) is a known predictor of clinical outcomes in STEMI patients, the possible association between characteristics of a large group of patients who undergo pPCI with LVEF and death was evaluated.

METHODS: This prospective cohort study included 304 patients who had undergone pPCI between 2009 and 2011. The association between LVEF and in-hospital outcomes of patients was assessed.

RESULTS: LVEF ≤ 25%, 25% < LVEF < 50%, and LVEF ≥ 50% were presented in 23 (7.6%), 150 (49.3%), and 128 (42.1%) of the patients, respectively. Three patients (0.01%) died before echocardiography. There was no significant difference among aforementioned three groups regarding baseline characteristics, except age (P = 0.012) and sex (P = 0.016). Cumulative number of cardiogenic shock and death were 7 (2.3%) and 22 (7.2%), respectively; with significant differences between three LVEF groups. Age more than 70 years old, pulmonary edema, systolic blood pressure < 100 mm Hg, shock, post-PCI thrombolysis in myocardial infarction (MI) flow grade, corrected thrombolysis in MI frame count, angiographic success and ST-segment resolution showed significant association with death (P < 0.050).

CONCLUSION: This study not only demonstrates that LVEF ≤ 50% is associated with a higher incidence of in-hospital adverse events, but also identifies characteristics that are strongly correlated with the risk of LVEF ≤ 50% and death after pPCI.

Keywords: Myocardial Infarction, Percutaneous Coronary Intervention, Ejection Fraction, Corrected Trombolysis in Myocardial Infarction

Introduction

Primary angioplasty is the best-known therapy for patients with ST-segment elevation myocardial infarction (STEMI) and for saving lives.[1] Sizable advancements in interventional techniques, equipments, and drugs coupled with better triage of patients have led to significant improvement in short and long-term clinical outcomes of STEMI patients. However, risk prediction in these patients remains problematic. Thereby, a practical prognostic criterion is needed. Since left ventricular ejection fraction (LVEF) is a known predictor of clinical outcomes in STEMI patients, the purpose of this study was to evaluate the possible association between demographical, clinical, and paraclinical characteristics of a large group of patients who undergo primary percutaneous coronary intervention (pPCI) with LVEF. Similarly, the association between demographic, clinical, and paraclinical characteristics of STEMI patients who have been found death was also reported.

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Materials and Methods

Participants and study design

This is a prospective cohort study conducted in Modarres Hospital (Tehran, Iran). A total of 304 patients with STEMI who underwent primary angioplasty during 2009-2011 were enrolled. STEMI was defined by the presence of ischemic chest discomfort within 12 h before hospital admission lasting for at least 20 min and associated with electrocardiographic criteria for STEMI.[2] Patients with prior thrombolytic therapy were excluded. Informed consent was obtained from all patients.

Procedure and assessment of variables

A checklist was filled out for all patients regarding baseline characteristics [age, gender, family history of coronary artery disease (CAD), smoking, diabetes mellitus, hypertension, dyslipidemia, renal insufficiency, prior aspirin usage, and prior CAD], physical examination on admission (systolic and diastolic blood pressure, heart rate, cardiogenic shock, and pulmonary edema), location of myocardial infarction (MI) [anterior (Ant.) MI vs. non-Ant. MI], door-to-balloon time, angiographic results, angiographic success rate, thrombolysis in myocardial infarction (TIMI) flow grade, corrected TIMI frame count (CTFC), ST-segment resolution, EF at discharge, and in hospital adverse events. Door-to-balloon time was defined as the interval between arrival to the hospital and the use of a therapeutic device such as thrombectomy device, balloon, or stent. Coronary angioplasty was performed in accordance to American College of Cardiology/American Heart Association guidelines, using the femoral approach, approved devices and techniques and in the presence of reduced TIMI flow grade < 3, and/or a culprit lesion stenosis of > 50%. Only the culprit lesion was targeted, and left ventriculography was not performed in none of patients. All coronary angiograms were reviewed by two interventional cardiologists who were blinded to all data apart from the angiograms; and TIMI flow grades and CTFCs were determined. All angiograms were performed with 7F guiding catheters. TFC is the number of cine frames needed for contrast to reach a standardized distal coronary landmark in the culprit vessel. TFC was determined by Gibson et al. method.[3] The first frame is selected when the column of the contrast extends across > 70% of the arterial lumen with antegrade flow. The number is expressed based upon a cine filming rate of 30 frames/s. The last frame is that in which the contrast enters the distal landmark. Distal landmark in the right coronary artery (RCA) is the first branch of the posterolateral extension of the RCA after the origin of the posterior descending artery; in the circumflex artery, it is the most distal branch of the obtuse marginal branch which includes the culprit lesion; and in the left anterior descending artery, it is the distal bifurcation which usually places at the apex of the heart. The CTFC means that the TFC for left anterior descending (LAD) must be corrected due to the longer length of the LAD by dividing it into 1.7.

Advised medical treatments were 325 mg of aspirin, 600 mg of clopidogrel, heparin, 20 mg of pantoprazole, and 40 mg of atorvastatin for all patients. The use of glycoprotein IIb/IIIa inhibitors, beta-blockers, enalapril or losartan, thrombectomy, intra-aortic balloon pump, and bare-metal or drug-eluting stents were left to the decision of the operators. Successful angioplasty of the infarct-related artery (IRA) was defined as sustained patency of the infarct-related vessel with TIMI III flow and < 50% final stenosis.

Electrocardiograms were recorded on arrival and 60 min after pPCI. ST resolution was measured 60 min after primary angioplasty at the same lead with maximal ST elevation in pre-angioplasty electrocardiogram. ST resolution > 70% was considered as a good result.

In-hospital adverse clinical events were cardiogenic shock, reinfarction, stent thrombosis, urgent target vessel revascularization (repeat PCI or coronary artery bypass grafting), major bleeding, cerebrovascular accident, need to dialysis, and death. Shock was diagnosed as persistent hypotension (systolic blood pressure < 90 mmHg) and associated signs of low cardiac output unresponsive to treatment. Reinfarction was defined as recurrence of clinical symptoms or development of new electrocardiographic changes accompanied with new elevation of creatine kinase MB enzyme levels. Ischemia-driven target vessel revascularization was any repeat PCI or coronary artery bypass surgery of the IRA prompted by clinical symptoms or objective evidence of ischemia. Major bleeding was defined either as intracerebral hemorrhage, a drop in the hemoglobin greater than 3 mg/dl, need for blood transfusions, or local bleeding requiring surgical treatment.

LVEF was evaluated before discharge, by 2D echocardiography based on Simpson's method. The study population was divided into three groups of LVEF ≤ 25% (severe LV systolic dysfunction), 25% < LVEF < 50% (moderate or mild LV systolic dysfunction), and LVEF ≥ 50% (preserved or normal LV systolic function).

Statistical analysis

Baseline characteristics were reported as mean ± standard deviation for continuous variables or percentages for categorical variables. Normality of the data for continuous variables was evaluated by Kolmogorov-Smirnov test. Then, continuous variables were compared by using a series of tests including ANOVA, and post-hoc Tukey for variables with normal distribution, and Kruskal-Wallis and Mann-Whitney for those without normal distribution. Categorical variables were evaluated by the chi-square test (or Fisher's exact test as needed). The 95% confidence intervals (CIs) for the odds ratio (OR) using multivariate logistic regression were calculated to measure the association between the patients' characteristics and the risk of in-hospital death. All analyses were performed by using the SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA). The P-value of less than 0.05 was considered to be statistically significant.

Results

The mean age of study population was 57.6 ± 11.1 (27-90) years, and 238 (78.3%) of all patients were male. Three patients (0.01%) died before echocardiography. Table 1 represents baseline characteristics of the enrolled patients stratified into three groups of LVEF ≤ 25%, 25% < LVEF < 50%, and LVEF ≥ 50%. There was a significant difference between the LVEF groups regarding age (P = 0.012) and sex (P = 0.016), though Tukey test revealed that the statistically significant difference was just observed between the two groups with LVEF ≤ 25% and 50% ≤ LVEF regarding age (P = 0.010). There was no significant difference between three groups of LVEF concerning coronary risk factors such as family history of CAD, smoking, diabetes mellitus, hypertension, dyslipidemia, and renal insufficiency (P > 0.050). Similarly, there was not any significant difference between three LVEF groups regarding hemodynamic findings (systolic, diastolic blood pressure, fraction of patients with systolic blood pressure less than 100 mm Hg, and heart rate). The aforementioned groups had significant differences in location of MI (Ant. MI vs. non-Ant. MI), presence of pulmonary edema, and shock (P < 0.000). In addition, door-to-balloon time was less than 60 min for all patients that revealed no significant difference between groups (P > 0.050).

Table 1. Baseline characteristics of patients who underwent primary percutaneous coronary intervention stratified by left ventricular ejection fraction

Characteristics	Left ventricular ejection fraction			P
	≤ 25%	25% < LVEF < 50%	≥ 50%	
Number of patients	23	150	128	-
Age ≥ 75 (year)	6 (26.1)	26 (17.3)	11 (8.6)	0.028
Gender, male	15 (65.2)	127 (84.7)	93 (72.7)	0.016
Family history of CAD	1 (4.3)	33 (22.0)	28 (21.9)	0.134
Current smoking	10 (43.5)	67 (44.7)	56 (43.8)	0.986
Dyslipidemia	7 (30.4)	50 (33.3)	43 (33.6)	0.956
Diabetes mellitus	5 (21.7)	35 (23.3)	30 (23.4)	0.984
Hypertension	12 (52.2)	69 (46.0)	43 (33.6)	0.060
Ant. MI vs. non Ant. MI	19 (82.6)	97 (64.7)	48 (37.5)	< 0.001
Shock	4 (17.4)	3 (2.0)	0 (0.0)	< 0.001
Pulmonary edema	6 (26.1)	3 (2.0)	0 (0.0)	< 0.001
Renal insufficiency	2 (8.7)	6 (4.0)	3 (2.3)	0.311
SBP < 100 (mmHg)	3 (13.0)	7 (4.7)	4 (3.1)	0.115
Prior aspirin usage	5 (21.7)	43 (28.7)	34 (26.6)	0.765
Prior coronary artery disease	1 (4.3)	7 (4.7)	5 (3.9)	0.953
Hemoglobin (mg/dl) (mean ± SD)	12.5 ± 1.7	13.1 ± 1.6	13.0 ± 1.7	0.359
Serum Cr (mg/l) (mean ± SD)	1.4 ± 0.9	1.2 ± 1.0	1.1 ± 0.3	0.052[*]
LDL (mg/dl) (mean ± SD)	96.4 ± 30.5	105.9 ± 25.0	102.7 ± 25.9	0.210
DBP (mm Hg) (mean ± SD)	73.9 ± 11.2	74.3 ± 7.6	74.2 ± 7.1	0.978
Pulse (beats/min) (mean ± SD)	80.8 ± 16.2	77.8 ± 15.4	74.0 ± 8.7	0.110[*]
SBP (mm Hg) (mean ± SD)	129.6 ± 28.5	123.7 ± 21.0	123.2 ± 16.9	0.371
Age (year) (mean ± SD)	63.5 ± 12.6	58.0 ± 11.6	56.2 ± 10.0	0.012

Values are presented as n (%) unless otherwise expressed; LVEF: Left ventricular ejection fraction; CAD: Coronary artery disease; Ant.: Anterior; MI: Myocardial infarction; SBP: Systolic blood pressure; SD: Standard deviation; Cr: Creatine; LDL: Low-density lipoprotein; DBP: Diastolic blood pressure
[*] Abnormal distribution was determined using Kruskal-Wallis test

Table 2 compares angiographic results of patients who underwent pPCI and stratified by LVEF. According to the angiographic findings, the number of narrowed vessels among these three LVEF groups showed significant difference (P < 0.001). Most number of narrowed coronary arteries were three, two, and single vessels in groups with LVEF ≤ 25%, 25% < LVEF < 50%, and LVEF ≥ 50%), respectively. Furthermore, the IRA was significantly different between the three groups (P < 0.001). The most IRA in patients with LVEF ≤ 25% and 25% < LVEF < 50% was the LAD artery, while for patients with LVEF ≥ 50% it was the RCA. All three LVEF groups, had similar initial TIMI flow grades (P = 0.473), but post-PCI TIMI flow grade showed significant difference (P = 0.013). The CTFC values in three groups with LVEF ≤ 25%, 25% < LVEF < 50%, and LVEF ≥ 50%, were 36.5 ± 35.2, 20.1 ± 15.5, and 18.2 ± 14.0, respectively; which was statistically significant (P < 0.000). Similarly, Tukey test revealed that significant difference was between patients with LVEF ≤ 25% and two other groups with 25% < LVEF < 50% and LVEF ≥ 50% (P < 0.001).

The angiographic success rate of pPCI in the three groups of LVEF (LVEF ≤ 25%, 25% < LVEF < 50%, and LVEF ≥ 50%) were 65.2%, 84.7%, and 89.1%, respectively, which revealed significant difference between groups (P = 0.013). Absence of ST-segment resolution in the mentioned groups was 47.8%, 17.3%, and 1.6%; respectively, which showed significant difference (P ≤ 0.001).

Table 3 compares in-hospital adverse events of patients who underwent pPCI and stratified by LVEF. In-hospital adverse clinical events did not have significant difference between groups, except for the gastrointestinal bleeding and death. Death rate in groups with LVEF ≤ 25%, 25% < LVEF < 50%, and LVEF ≥ 50% was 30.4%, 7.3%, and 0.8%, respectively (P < 0.001).

Table 4 represents OR of patients' characteristics associated with the risk of in-hospital death in patients who underwent pPCI. Characteristics that showed significant association with death include age more than 70 years old, pulmonary edema, systolic blood pressure < 100 mmHg, shock, post-PCI TIMI flow grade, CTFC, angiographic success, and ST-segment resolution (P < 0.050).

Table 2. Angiographic results of patients who underwent primary percutaneous coronary intervention stratified by left ventricular ejection fraction

Characteristics	Left ventricular ejection fraction			P
	≤ 25%	25% < LVEF < 50%	≥ 50%	
Number of patients	23	150	128	-
Number of narrowed vessels	-	-	-	
One vessel disease	2 (8.7)	50 (33.3)	79 (61.7)	
Two vessel disease	8 (34.8)	66 (44.0)	42 (32.8)	< 0.001
Three vessel disease	13 (56.5)	32 (21.3)	6 (4.7)	
Left main involvement	0 (0.0)	2 (1.3)	1 (0.8)	
Infarct-related artery	-	-	-	
LAD	19 (82.6)	96 (64.0)	49 (38.3)	
RCA	4 (17.4)	41 (27.3)	69 (53.9)	< 0.001
LCX	0 (0.0)	13 (8.7)	10 (7.8)	
SVG	0 (0.0)	0 (0.0)	0 (0.0)	
Initial TIMI flow grade ≤ 1	23 (100.0)	141 (94.0)	120 (93.8)	0.473
Post-PCI TIMI flow grade < 3	8 (34.8)	23 (15.3)	14 (10.9)	0.013
CTFC > 20	11 (47.8)	46 (30.7)	31 (24.2)	0.062
Intra-aortic balloon pump	8 (34.8)	8 (5.3)	0 (0.0)	< 0.001
Angiographic success	15 (65.2)	127 (84.7)	114 (89.1)	0.013
Stent treatment	20 (87.0)	147 (98)	124 (96.9)	0.450
No ST-segment resolution	11 (47.8)	26 (17.3)	2 (1.6)	< 0.001
CTFC (mean ± SD)	36.5 ± 35.2	20.1 ± 15.5	18.2 ± 14.0	< 0.001
Contrast volume (ml) (mean ± SD)	345.7 ± 132.6	319.5 ± 75.1	311.6 ± 80.6	0.187

Values are presented as n (%) unless otherwise expressed; LVEF: Left ventricular ejection fraction; LAD: Left anterior descending LCX: Left circumflex; RCA: Right coronary artery; SVG: Saphenous vein graft; TIMI: Thrombolysis in myocardial infarction; CTFC: Corrected thrombolysis in myocardial infarction frame count

Table 3. In-hospital complications of patients who underwent primary percutaneous coronary intervention stratified by left ventricular ejection fraction

Characteristics	Left ventricular ejection fraction			P
	≤ 25%	25% < LVEF < 50%	≥ 50%	
Number of patients	23	150	128	-
Reinfarction	0 (0.0)	1 (0.7)	0 (0.0)	0.603
Stent thrombosis	0 (0.0)	0 (0.0)	0 (0.0)	-
Repeat PCI	1 (4.3)	3 (2.0)	0 (0.0)	0.147
CABG	1 (4.3)	6 (4.0)	0 (0.0)	0.070
Gastrointestinal bleeding	1 (4.3)	0 (0.0)	0 (0.0)	0.002
Dialysis	0 (0.0)	0 (0.0)	0 (0.0)	-
Cerebrovascular accident	0 (0.0)	0 (0.0)	0 (0.0)	-
Death	7 (30.4)	11 (7.3)	1 (0.8)	< 0.001
Hospital stay, days (mean ± SD)	6.3 ± 4.9	5.9 ± 0.2	5.9 ± 4.3	0.858

Values are presented as n (%) unless otherwise expressed; LVEF: Left ventricular ejection fraction; PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass grafting

Table 4. Odds ratio of characteristics associated with the risk of death in patients who underwent primary percutaneous coronary intervention

Characteristics	Live	Death	Odds ratio	95% Confidence interval	P
Number of patients	282	22	-	-	-
Age ≥ 70 years	35 (12.4)	8 (36.4)	0.2	0.1-0.6	0.002
Gender, male	222 (78.7)	16 (72.7)	0.7	0.3-1.9	0.511
Hypertension	112 (39.7)	13 (59.1)	2.2	0.9-5.3	0.075
Diabetes mellitus	67 (23.8)	3 (13.6)	0.5	0.1-1.8	0.277
Pulmonary edema	3 (1.1)	6 (26.6)	34.9	8.0-152.4	< 0.001
Ant. MI vs. non-Ant. MI	151 (53.5)	15 (68.2)	1.9	0.7-4.7	0.184
LAD as IRA	154 (54.6)	12 (54.5)	1.0	0.4-2.4	0.995
SBP < 100 mmHg	7 (2.5)	8 (36.4)	16.0	5.1-50.0	< 0.001
Shock	2 (0.7)	5 (23.8)	41.2	7.4-228.0	< 0.001
Intra-aortic balloon pump	7 (2.5)	11 (50.0)	39.3	12.8-120.8	< 0.001
Initial TIMI flow grade ≤ 1	265 (94.0)	22 (100.0)	0.9	0.9-1.0	0.236
Post-PCI TIMI flow grade < 3	33 (11.7)	13 (59.1)	10.9	4.3-27.5	< 0.001
CTFC > 20	73 (25.9)	18 (81.8)	0.1	0.03-0.2	< 0.001
Angiographic success	249 (88.3)	9 (40.9)	10.9	4.3-27.5	< 0.001
No ST-segment resolution	28 (9.9)	13 (59.1)	0.1	0.03-0.2	< 0.001

Values are presented as n (%); Ant.: Anterior, MI: Myocardial infarction; LAD: Left anterior descending; IRA: Infarct-related artery; SBP: Systolic blood pressure; TIMI: Thrombolysis in myocardial infarction; CTFC: Corrected thrombolysis in myocardial infarction frame count

Discussion

The mean age of the overall study population was lower (< 60 years) than previous studies run in this area (> 60 years), whereas the percentage of patients who were older than 75 years old was high (14.1%).[4] The elderly patients (> 75 years old) with their higher mortality rate versus younger patients (18.6% vs. 5.4%, respectively), increased the rate of total mortality. The mortality rate in older patients was equal to studies from developed countries.[5] Other baseline characteristics of the enrolled patients were similar to previous studies.[6,7] Compatible with two new studies, sex showed significant difference between LVEF groups.[8,9] Though death rate in females and males was 9.1% and 6.7%, respectively;

however, it was not statistically significant, which was probably due to insufficient number of patients.

Incidence of pulmonary edema was similar to recent studies and heart failure was a strong predictor of death in this study [OR: 34.9 (95% CI: 8.0-152.4), P < 0.001].[10]

Angiographic findings according to number of narrowed vessels, IRA, and initial TIMI flow grade ≤ 1 were similar to recent reports.[11,12]

The TIMI flow grading system is a qualitative method for evaluation of reperfusion. Furthermore, TIMI flow grade < 3 after pPCI is associated with increased incidence of major in-hospital adverse events.[13] The CTFC is a quantitative method for measuring reperfusion. The mean CTFC in the normal coronary arteries is 21.1 ± 1.5 for LAD,

22.2 ± 4.4 for left circumflex, 20.4 ± 3.3 for RCA.[3] The CTFC is an independent predictor of prognosis and death following STEMI. In our previous study, CTFC of the IRA did not have significant association with LVEF in STEMI patients who underwent pPCI.[14] However, current study with more enrolled patients showed significant association between CTFC values and LVEF. Although, the percentages of patients with CTFC > 20 did not have significant association with LVEF (P = 0.062), but a larger study population seems more suitable and necessary.

In patients with STEMI, ST-segment resolution results in a better global LVEF, which in turn leads to a survival benefit.[15]

Reported failure rate is 4-11% for pPCI.[16] Failure of PCI in STEMI patients is associated with poor outcomes.[17] In this study, angiographic success was associated with better LVEF and lower mortality rates.

The in-hospital mortality rate in this study was 7.2%, which was similar to a number of newer studies and less than older studies.[4,5,18,19] Death can be predicted from baseline, clinical, and angiographic characteristics of the patients. Hence, these high risk patients will be triaged for more intensive observation and treatment. Therefore, in order to achieve more sound decisions, every population in each country needs its own data for better judgments in clinical situations.

Study limitations

This study has some limitations. First, data was obtained from a single hospital, so external validation is necessary. Second, long-term follow-up data are needed for thorough analysis and post-discussions thereby the author(s) made their best to publish follow-up data in the second manuscript. Third, the repeat study including larger number of patients will help to better analysis and achievement of more reliable results. Overestimation of the ORs in this study with moderate sample size is probably.

Conclusion

LVEF is an independent predictor of all-cause death in patients who undergone pPCI. Patients with older age, female gender, anterior MI, higher heart rate, pulmonary edema, shock, need to IABP, post-PCI TIMI flow grade < 3, higher CTFC, and absent angiographic success or ST-segment resolution after pPCI had significantly higher rates of low LVEF. Awareness of these predictors may assist clinicians to make better clinical decisions for STEMI patients and to facilitate possible future research.

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www.mui.ac.ir   9 Jul

Relationship between blood peroxidases activity and visfatin levels in metabolic syndrome patients

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Original Article

Abstract

BACKGROUND: The observed relationships between visfatin, peroxidases activity, and metabolic syndrome (MetS) are inconsistent; therefore, this study was undertaken to understand these relationships.

METHODS: This cross-sectional study was conducted as a part of the Isfahan Healthy Heart Program, Iran. A blood sample of 90 MetS and non-MetS patients were used to estimate total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), fasting blood glucose (FBG), waist circumference (WC), systolic blood pressure (SBP) and diastolic blood pressure (DBP), visfatin and peroxidases activity. Data analysis for MetS group was carried out in two ways. (1) MetS with three components and with > 3 components. (2) MetS with hyperglycemia and without hyperglycemia.

RESULTS: SBP, DBP, WC, FBG, TC, TG, LDL-C, and were higher and HDL-C levels was lower in MetS patients. There was a significant correlation between visfatin levels and peroxidases activity in MetS patients with three components. Levels of visfatin were significantly higher in male as compared to female subjects in the MetS with three components group. There was a significant decrease in peroxidases activity in > 45 years old subjects in the MetS with > 3 components group. A significant correlation was observed between serum visfatin levels and FBG in the MetS without hyperglycemia group.

CONCLUSION: Peroxidases activities in MetS patients can be related to visfatin levels. Gender influences on peroxidases activity probably and was lower in female patients with MetS. Hyperglycemia does not influence peroxidases activities and visfatin levels.

Keywords: Peroxidase, Metabolic Syndrome, Visfatin

Introduction

The prevalence of metabolic syndrome (MetS) has increased in recent decades,[1] and has been described as a cluster of multiple, partially or fully expressed, metabolic abnormalities within the single individual that increase the risk of developing cardiovascular disease and diabetes.[2,3] In recent years, there has been much interest in the role of free radicals and oxidative stress in the pathogenesis of MetS.[4] It has been shown that obesity per se may induce systemic oxidative stress and that increased oxidative stress in accumulated fat is, at least in part, the underlying cause of the dysregulation of adipocytokines and the development of MetS.[5] Adipocytokines include adiponectin, leptin, resistin, and visfatin that are secreted from adipose tissue.[6]

In human pulmonary vascular endothelial cells, visfatin was demonstrated to interact with several proteins mediating oxidative stress and inflammation leading to increased levels of reactive oxygen species.[7] Oxidative stress may be defined as

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an imbalance between the production and degradation of reactive oxygen species. Enzymatic inactivation of reactive oxygen species is achieved mainly by antioxidative enzymes.[8] The main antioxidant enzymes are glutathione peroxidase (GPx), superoxide dismutase, catalase, and myeloperoxidase.[9] Peroxidases are a family of widespread enzymes which perform distinct tasks. On one hand, they act as preventive antioxidants to detoxify damaging lipid peroxides or other peroxides from blood and organic substrates. On the other hand, these enzymes function as starters for oxidative reactions, thereby generating a source of reactive oxygen species such as hypochlorous acid (HOCl) or hypoiodous acids (HOI).[10]

Increasing the visfatin levels can be observed in atherosclerosis,[11] endothelial dysfunctions,[12,13] and renal insufficiency.[14] Evidence on possible associations between serum visfatin and metabolic parameters in patients with obesity and diabetes are contradictory.[14-18] Takebayashi et al.[14] did not find any correlation between visfatin and diabetes, and other study proved that there is a positive correlation between the decrease of visfatin and type 1 diabetes and negative correlation between glycated hemoglobin and visfatin levels.[19] Berndt et al.[16] and Hammarstedt et al.[20] have reported that serum concentration of visfatin is increased in obesity. On the other hand, Pagano et al.[21] revealed that plasma visfatin was significantly lower in obese subjects.

The effect of hyperglycemia on levels of visfatin is discussed. Alexiadou et al.[22] finding was discordance with previous study[23] demonstrating that visfatin is enhanced by hyperglycemia.

The visfatin levels and peroxidases activity are important in MetS, whereas only very few studies have been conducted to clarify the relationships between visfatin and peroxidases, and these factors and MetS. Therefore, the present study was designed to understand these relationships.

Materials and Methods

Participants

This cross-sectional study was conducted in 2012 as a part of the Isfahan Healthy Heart Program, Iran, (IHHP). IHHP began in 2000 to prevent and control cardiovascular disease risk factors in the Iranian population. This program was conducted in Central Iran. A stratified multi-stage probability sampling method was used in the baseline survey (2001) and the post-intervention in 2007.[24]

Blood samples (from 90 subjects within the age range of 19-82 years) of IHHP third phase (2006-2007) were used for this study. Samples of subjects with MetS (n = 45) and also without MetS (n = 45), were selected using simple random sampling. MetS defined by the National Cholesterol Education Program Adult Treatment Panel III as the presence of 3 or more of the following criteria: abdominal obesity: waist circumference (WC) ≥ 102 cm in men and ≥ 88 cm in women and 2 or more of the following: systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg; triglyceride (TG) ≥ 150 mg/dl; high-density lipoprotein cholesterol (HDL-C) < 40 mg/dl in men and < 50 mg/dl in women; fasting blood glucose (FBG) ≥ 110 mg/dl.[10] The study protocol was approved by the Medical Ethics Committees of the Isfahan Cardiovascular Research Institute under the Approval No. 91115.

Biochemical and anthropometric measurement

All measurements were conducted using calibrated instruments and standard protocols by a trained team of general physicians and nurses. Blood samples were collected from both groups to measure the biochemical factors following a 12-h fasting. All the blood sampling procedures were performed in the central laboratory of the Isfahan Cardiovascular Research Institute. FBG and serum lipids, including serum total cholesterol (TC), TG and HDL-C levels were detected by an enzymatic method using an Elan 2000 auto analyzer (Ependorf, Hamburg, Germany). Low-density lipoprotein-cholesterol (LDL-C) was calculated (in serum samples with TG ≤ 400 mg/dl) according to the Friedewald formula.[25]

The separated serum was stored at −70 °C until the measurement of visfatin levels. Visfatin levels were assayed by ELISA kit (BioVendor Laboratory Medicine Inc., Canada and Mexico, USA). Determination of peroxidases activity in the serum was done by the reaction of endogenous peroxidases with hydrogen peroxide, using 3,5,3',5'-tetramethylbenzidine as the chromogenic substrate.[26] A mercury sphygmomanometer with a cuff size suitable for each subject was used for measuring sitting blood pressure twice from the right arm according to World Health Organization criteria. The mean of two measurements of korotkoff phase I and phase IV was recorded for SBP and DBP, respectively. WC was determined from the point halfway between the lower border of ribs and the iliac crest in a horizontal plane.[27]

Statistical analysis

Statistical analyses were performed using SPSS for

Windows (version 15; SPSS Inc., Chicago, IL, USA). Data were presented as means ± standard deviation. Data analysis for MetS group was carried out in two ways: (1) MetS with three components and with > 3 components. (2) MetS with hyperglycemia and without hyperglycemia. Analysis of covariance was used to compare factors between groups with adjusting age and sex. Also for significant differences, Bonferroni multiple comparison was applied. For comparing visfatin levels and peroxidases activity in groups based on age and sex (without adjustment) Kruskal-Wallis test was used. Mann-Whitney tests with Bonferroni adjustment used to multiple comparisons. Investigation of correlation between visfatin levels and peroxidases activity and also between these two factors with parameters of MetS was assessed using spearman and partial correlation. P-value of < 0.05 was considered to be statistically significant.

Results

Findings on the values of the biochemical factors are summarized in table 1. The MetS subjects had significantly higher values of SBP, DBP, WC, FBG, TC, TG, LDL-C, and lower levels of HDL-C than the non-MetS subjects. There was no significant difference in serum levels of visfatin and peroxidases activity between MetS and non-MetS groups.

Table 2 shows the correlation between visfatin levels and peroxidases activity in the studied groups. There was a significant correlation between visfatin levels and peroxidases activity in MetS subjects with three components whilst non-significant correlation was observed between these factors in the other groups (non-MetS, MetS with > 3 components, and MetS with and without hyperglycemia).

Changes in visfatin levels and peroxidases activity based on sex and age were compared between non-MetS, MetS with three components, and MetS with > 3 components (Table 3) and between non-MetS, MetS with hyperglycemia and MetS without hyperglycemia (Table 4). There was no significant difference in serum visfatin levels between MetS and non-MetS groups in sex and age groups. For peroxidases activity, there was significant difference between non-MetS and MetS with three components groups in female subjects and also between MetS with three components and with > 3 components groups in 19-44 years old subjects. Peroxidases activity did not significantly changed between non-MetS, MetS with

Table 1. Demographic, clinical, and laboratory variables adjusted for age and sex in patient with and without metabolic syndrome

Characteristics	Non-MetS (n = 45)	MetS with three components (n = 29)	MetS with > 3 components (n = 16)	P	Non-MetS (n = 45)	MetS with hyperglycemia (n = 17)	MetS without hyperglycemia (n = 28)	P
Visfatin (mg/ml)	2.83 ± 3.43	3.02 ± 3.27	3.17 ± 3.47	0.944	2.83 ± 3.43	3.07 ± 2.10	3.07 ± 2.61	0.464
Peroxidase activity (mU/ml)	19.23 ± 30.03	3.76 ± 27.91	7.77 ± 30.03	0.050	20.03 ± 30.16	8.17 ± 18.42	8.17 ± 22.93	0.191
Total cholesterol (mg/dl)	180.57 ± 34.72**	207.08 ± 32.28*	195.87 ± 34.74	0.006	179.98 ± 34.59**	209.20 ± 33.22*	200.00 ± 33.12	0.007
Triglycerides (mg/dl)	100.19 ± 95.92**,€	160.08 ± 89.27*,€	261.18 ± 96.01**,***	0.001	106.20 ± 10.17**,€	219.00 ± 9.79*	174.74 ± 9.74*	0.001
High-density lipoprotein cholesterol (mg/dl)	46.97 ± 9.74€	41.86 ± 9.01€	33.07 ± 9.75*,***	0.001	46.49 ± 101.35€	40.83 ± 97.49	38.00 ± 97.15*	0.004
low-density lipoprotein cholesterol (mg/dl)	113.39 ± 29.41**	132.54 ± 27.40*	113.54 ± 29.44	0.008	112.31 ± 30.16	127.33 ± 20.03*	126.08 ± 28.89*	0.049
Fasting blood glucose (mg/dl)	84.21 ± 18.98€	91.83 ± 17.63	104.64 ± 19.00*	0.003	85.09 ± 16.04**	112.81 ± 15.42*,€	85.16 ± 15.36*,***	0.001
Systolic blood pressure (mmHg)	114.12 ± 17.86**,€	125.48 ± 16.31*	130.42 ± 17.39*	0.005	114.42 ± 17.86**,€	127.66 ± 12.56*	126.70 ± 16.59*	0.007
Diastolic blood pressure (mmHg)	73.32 ± 11.36*,€	82.80 ± 10.39*	85.63 ± 11.07*	0.001	73.50 ± 11.30*,€	85.18 ± 10.82*	82.82 ± 10.52*	0.001
Waist circumference (cm)	88.37 ± 11.86**	97.00 ± 10.85*	92.93 ± 11.45	0.009	88.12 ± 11.92**	96.68 ± 11.22*	95.05 ± 11.02	0.016

The results are expressed as mean values ± standard deviation (SD); P-values are significant P < 0.05; * Significant difference with non-MetS; ** Significant difference with MetS with three components or with > 3 components; *** Significant difference with MetS with > 3 components or without hyperglycemia; €Significant difference with MetS with hyperglycemia; Analysis of covariance was used; MetS: Metabolic syndrome

hyperglycemia and MetS without hyperglycemia in sex and age groups. Levels of visfatin were significantly higher in male subjects than female in the MetS with three components. There was significant reduction in peroxidases activity in > 45 years old subjects in comparison with 19-44 years old subjects in the MetS with > 3 components.

Correlation between visfatin levels and peroxidases activity with components of MetS are provided in table 5. There was no significant correlation between serum visfatin levels and lipid profile, FBG, SBP, DBP, and WC in MetS and non-MetS groups except visfatin and FBG in the MetS without hyperglycemia subjects. No statistically significant correlation was found between peroxidases activity and studied factors.

Table 2. Correlation between visfatin levels and peroxidase activity in two non-metabolic syndrome and metabolic groups

Biochemical factors	Groups	Spearman's correlation with visfatin levels (mg/ml)	P
Peroxidase activity (mU/ml)	Non-MetS (n = 45)	0.094	0.581
	MetS with three components (n = 29)	0.769	0.001
	MetS with > 3 components (n = 16)	0.315	0.253
	MetS with hyperglycemia (n = 17)	−0.244	0.328
	MetS without hyperglycemia (n = 28)	0.189	0.345

P-values are significant P < 0.05; Spearman correlation was used; MetS: Metabolic syndrome

Table 3. Visfatin levels and glutathione peroxidase activity based on sex and age in non-metabolic syndrome and metabolic syndrome (with three components, with > 3 components of metabolic syndrome) groups

Variable	Non-metabolic syndrome (n = 45)	Metabolic syndrome with three components (n = 29)	Metabolic syndrome with > 3 components (n = 16)	P
Visfatin (mg/ml)				
Sex				
Female (n = 49)	2.80 ± 3.51	1.70 ± 1.10	3.14 ± 2.41	0.206
Male (n = 41)	2.85 ± 2.17	4.33 ± 5.12	3.00 ± 1.76	0.841
P	0.435	0.022	0.842	
Age				
19-45 year (n = 56)	2.93 ± 3.29	3.11 ± 4.52	2.80 ± 2.94	0.963
> 45 year (n = 34)	2.40 ± 2.09	2.73 ± 2.84	3.16 ± 1.58	0.277
P	0.716	0.968	0.389	
Peroxidase activity (mU/ml)				
Sex				
Female (n = 49)	20.46 ± 35.35[**]	1.64 ± 1.53[*]	12.57 ± 28.89	0.043
Male (n = 41)	23.71 ± 41.90	4.21 ± 6.80	14.23 ± 30.76	0.376
P	0.314	0.060	0.272	
Age				
19-45 year (n = 56)	22.91 ± 37.80	3.72 ± 6.58[£]	43.06 ± 45.31[**]	0.022
> 45 year (n = 34)	16.62 ± 37.38	1.90 ± 1.24	2.03 ± 1.67	0.102
P	0.598	0.853	0.005	

The results are expressed as mean values ± standard deviation (SD); P-values are significant P < 0.05; Kruskal-Wallis test and Mann-Whitney tests (for multiple comparison) was used; [*] Significant difference with non-MetS; [**] Significant difference with MetS with three components or with hyperglycemia; [£] Significant difference with MetS with > 3 components or without hyperglycemia

Table 4. Visfatin levels and glutathione peroxidase activity based on sex and age in non-metabolic syndrome and metabolic syndrome (with hyperglycemia and without hyperglycemia) groups

Variable	Non metabolic syndrome (n = 45)	Metabolic syndrome with hyperglycemia (n = 17)	Metabolic syndrome without hyperglycemia (n = 28)	P
Visfatin (mg/ml)				
Sex				
Female	2.80 ± 3.51	2.00 ± 1.27	2.25 ± 2.05	0.838
Male	2.85 ± 2.17	2.50 ± 1.60	4.41 ± 4.79	0.778
P	0.435	0.489	0.065	
Age				
19-44 years	2.93 ± 3.39	1.66 ± 1.32	4.00 ± 5.16	0.103
> 45 years	2.40 ± 2.09	2.63 ± 2.36	3.12 ± 2.84	0.399
P	0.716	0.078	0.804	
Peroxidase activity (mU/ml)				
Sex				
Female	20.46 ± 35.35	4.83 ± 15.65	8.87 ± 22.12	0.052
Male	23.71 ± 41.90	1.79 ± 1.64	9.28 ± 23.78	0.581
P	0.314	0.077	0.108	
Age				
19-44 years	22.91 ± 37.80	5.38 ± 8.67	16.89 ± 32.63	0.514
> 45 years	16.62 ± 37.38	1.95 ± 1.63	1.97 ± 1.34	0.052
P	0.598	0.394	0.333	

The results are expressed as mean values ± standard deviation (SD); P-values are significant $P < 0.05$; Kreskas-Wallis test and Mann-Whitney tests (for multiple comparison) was used

Table 5. Correlation of visfatin levels and glutathione peroxidase activity adjusted for age and sex with parameters of metabolic syndrome

Characteristic	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	FBG (mg/dl)	SBP (mmHg)	DBP (mmHg)	WC (cm)
Visfatin (mg/ml)								
Non-MetS (n = 45)	−0.078	−0.056	−0.198	−0.013	0.036	−0.027	0.054	0.094
P	0.648	0.740	0.240	0.937	0.832	0.879	0.759	0.596
MetS with three components (n = 29)	0.193	0.291	−0.034	0.046	−0.348	0.134	0.058	0.257
P	0.308	0.118	0.857	0.809	0.060	0.489	0.766	0.186
MetS with > 3 components (n = 16)	−0.321	0.198	0.054	−0. 007	−0.100	0.284	0.448	−0.231
P	0.458	0.480	0.848	0.979	0.722	0.305	0.094	0.408
MetS with hyperglycemia (n = 17)	0.286	0.386	−0.144	−0.002	0.151	0.221	0.338	0.373
P	0.250	0.113	0.570	0.994	0.549	0.395	0.185	0.186
MetS without hyperglycemia (n = 28)	0.175	0.327	−0.010	−0.015	−0.512	0.145	0.065	−0.032
P	0.382	0.096	0.960	0.939	0.006	0.469	0.749	0.876
Peroxidase activity (mU/ml)								
Non-MetS (n = 45)	−0.117	−0.206	−0.184	−0.033	−0.220	−0.183	−0.256	−0.130
P	0.491	0.221	0.277	0.847	0.190	0.292	0.138	0.464
MetS with three components (n = 29)	−0.093	−0.093	0.084	−0.086	−0.302	0.058	−0.002	0.024
P	0.617	0.618	0.655	0.646	0.099	0.759	0.991	0.903
MetS with > 3 components (n = 16)	−0.321	−0.313	−0.070	0.012	0.164	0.168	0.106	−0.037
P	0.225	0.238	0.796	0.966	0.543	0.535	0.696	0.890
MetS with hyperglycemia (n = 17)	−0.291	−0.363	−0.007	0.069	0.169	−0.082	−0.367	−0.117
P	0.241	0.138	0.980	0.784	0.503	0.755	0.147	0.653
MetS without hyperglycemia (n = 28)	−0.113	0.071	−0.204	−0.114	−0.247	−0.183	−0.256	0.062
P	0.560	0.715	0.289	0.555	0.197	0.292	0.138	0.755

P-values are significant $P < 0.05$; Partial correlation was used; MetS: Metabolic syndrome; TC: Total cholesterol; TG: Triglycerides; HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol; FBG: Fasting blood glucose; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; WC: Waist circumference

Discussion

The findings of the current study provide evidence-based information about the impacts of visfatin levels and peroxidases activity on parameters of MetS. There was positive correlation between visfatin levels and peroxidases activity in MetS subjects with three components.

Peroxidases activity was higher in MetS with three components than the non-MetS in people female subjects. Peroxidases activity reduced with increasing age in the MetS with > 3 components group and visfatin levels enhanced in male subjects in the MetS with three components group.

Activities of antioxidant enzymes protect against oxidative stress in MetS.[28] Oxidative stress is associated with many of the components of the syndrome, leading to the concept that the amelioration of risk factors comprising MetS, including insulin resistance, elevated blood pressure, elevated lipid levels, inflammation and endothelial dysfunction may ameliorate oxidative stress and thus curtail the progression of metabolic disease complications.[29]

The results by Vavrova et al.[28] implicated an increased oxidative stress in MetS and a decreased antioxidative defense that correlated with some laboratory (TG, HDL-C) and clinical (WC, BP) components of MetS.

Here, we showed a higher serum visfatin levels in patients with MetS however this elevation was no significant. Consistent with our findings, studies have shown elevated serum visfatin levels patients with MetS when compared to individuals without MetS.[30-32] Primary investigation on visfatin showed the insulin-mimicking effect of this hormone.[33] Hence, one would conclude that an elevated visfatin levels in patients with MetS is due to insulin resistance. Cekmez et al.[34] suggested visfatin as a marker of insulin resistance. Inconsistently, Esteghamati et al.[32] showed a higher visfatin concentration, independent of insulin resistance, in type 2 diabetes. Furthermore in two other separate studies, Berndt et al.[16] and Haider et al.[35] showed that visfatin levels were not correlated with insulin resistance and lipid parameters in patients with type 2 diabetes and obesity. A study by Kaminska et al.[36] revealed elevated levels of visfatin in obese subjects did not correlate with the majority of anthropometric parameters. They suggested that elevated visfatin levels are associated with the distribution of adipose tissue characteristic of gynoid rather than visceral obesity.

Yen et al.[37] reported, the subjects suffering from

MetS might be under higher oxidative stress, resulting in low levels of antioxidant enzyme activities. MetS is a type of metabolic disorder rather than a disease. Subjects with MetS might be under higher oxidative stress; antioxidant enzymes are the first line of defense against reactive oxygen species and may decrease to adjust to higher levels of oxidative stress.[38] In addition, MetS subjects in general were typically abdominally obese. Obesity is also an oxidative burden that may lead to the reduction of antioxidant enzymes activities.[39] Oxidative stress associate with advancing age.[40] Therefore, the findings of our study confirm the previous study's results.

Mecocci et al.[41] concluded that senescence seems be associated with a decline in nutritional antioxidants together with an increase in antioxidant enzyme activity; the latter understood as an adaptive response to an increased level of oxidation products.

In our study, peroxidase activity decreased with age increase in all of groups, especially MetS with > 3 components.

Because inhibition of cholesteryl ester transfer protein increases HDL-C level and decreases LDL levels,[42,43] one explanation of visfatin in cholesterol homeostasis may be via inhibition of cholesteryl ester transfer protein. The sex difference of correlation between visfatin and cholesterol levels may be due to estrogen effect. Estrogen may modulate visfatin to inhibit cholesteryl ester transfer protein in cholesterol homeostasis.[44]

Some studies had examined the relationship between plasma visfatin concentration and age in different populations. However, the results were inconsistent. A negative correlation was found in women with gestational diabetes mellitus,[45] but a positive correlation in patients with MetS.[46] The obtained results by Dogru et al.[15] were consistent with this study.

Decrease of oxidative stress association with elevating the expression of antioxidant enzymes, superoxide dismutase, catalase, glutathione, and GPx in addition to lowering LDL-C, TG, and CRP and elevating HDL-C.[47] Chen et al.[48] reported the value of WC was significantly correlated with and GPx activities in MetS patients.

We did not find any correlations between visfatin and lipid profile, glucose, and other measured parameters. Our results are different from previous reports. Contrary to our results, in multiple step-wise regressions analysis by Zhong et al.[49] LDL-C was identified as the independent factor that

influences serum visfatin. They concluded visfatin may correlate with the metabolism of cholesterol. Furthermore in the study by Chen et al.,[45] serum visfatin correlated negatively with LDL-C in women with MetS. Fukuhara et al.[33] identified visfatin as an adipocytokine predominantly secreted from visceral adipocytes. Computed tomographic scan demonstrated that plasma visfatin levels correlated strongly with the visceral fat area and weakly with the subcutaneous fat area in 101 male and female human subjects.[33] One of the study revealed visfatin levels correlate with WC and waist-hip ratio.[44] However, previous reports[16,49,50] and this study have not found this correlation. The discrepancy between the studies may be explained by differences in patient populations or different methods of sample collection[51] and detection.[52]

Conclusion

Peroxidases activities in MetS patients can be related to visfatin levels. Gender influences on GPx activity probably and was lower in female patients with MetS. Hyperglycemia does not influence peroxidases activities and visfatin levels.

Suggestions

Further study needs to be done to clarify the exact role of visfatin in MetS, especially homeostasis of lipid. According to the menstrual cycle influences on levels of visfatin and peroxidases activity and thus it should be considered. The correlation between other antioxidant enzymes such as superoxide dismutase, catalase, and glutathione with visfatin is investigated in the future.

Study limitations

Our study had some limitations. First, the number of participants of each both groups was small. Second, this study was a cross-sectional study, and therefore, no causal relationship could be defined. Third, age range of the participants was wide that may be influencing on peroxidases activity and plasma visfatin levels.

Acknowledgments

Conflict of Interests

Authors have no conflict of interests.

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Protection against ischemia-reperfusion injury in prolonged resuscitation: A case report and review of literature

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Case Report

Abstract

BACKGROUND: The severity of ischemia/reperfusion injury determines the neurologic outcome after successful cardiopulmonary resuscitation.

CASE REPORT: We present a case of prolonged open-chest resuscitation who survived without neurologic sequel. Multiple applied strategies to limit the deleterious effects of ischemia and reperfusion injury, that is, infusion of magnesium sulfate and mannitol, protective lung ventilation and optimal postoperative pain control prevented the end organ damage in this patient. During the 40 min open-chest resuscitation, ventricular defibrillation was successfully attempted with extrathoracic paddles.

CONCLUSION: The appropriate use of pharmacologic and non-pharmacologic protective strategies could modify the inflammatory cascade and minimize the deleterious effects of reperfusion after prolonged periods of ischemia. The successful defibrillation in this patient warrants the use of standard paddles in open-chest surgeries where surgical small paddles are not available.

Keywords: Resuscitation, Ischemia, Reperfusion, Neuroprotection, Addiction, Extrathoracic Defibrillation

Introduction

The successful cardiopulmonary-cerebral resuscitation requires intensive care to prevent end organ damage or neurologic sequel. Multiple pharmacologic strategies have been proposed to protect vital organs from ischemia/reperfusion injury.[1] However, their overall clinical benefit is controversial. Since conducting clinical trials addressing human resuscitation is difficult, and the clinical pictures are highly variable, any evidence for the effectiveness of a treatment modality even in a single patient would be valuable. In this report, the treatments with possible protective mechanisms have been explained.

Case Report

A 27-year-old man was emergently transported to the operating room due to hemorrhagic shock and cardiac tamponade following penetrating chest trauma. The patient was confused, with a heart rate of 134/min and blood pressure (BP) of 72/34. Induction of anesthesia was performed with ketamine 30 mg and succinylcholine 70 mg, and the patient intubated.

The surgeon approached the patient with an anterolateral thoracotomy. Immediately after pericardiotomy the patient became asystole. Open cardiac massage was started for the patient and continued for about 40 min, when electrocardiographic monitoring revealed ventricular fibrillation. Amiodarone 300 mg was slowly infused for the patient. Because internal paddles were not available, extrathoracic defibrillation with monophasic shock 200 J using standard paddles was attempted. The cardiac rhythm immediately changed to sinus rhythm with a BP of 102/47. Magnesium sulfate 2 g was slowly infused for the patient. The BP remained stable in the remaining time of the operation. The stab wound (approximately 2 cm) in the apex of the left ventricle was sutured, pericardium was closed, chest tube was inserted, the ribs were approximated and finally chest wall was closed.

During the 40 min cardiopulmonary resuscitation (CPR) the patient received only a small

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dose of hyoscine (5 mg) to guarantee his amnesia. From successful defibrillation to the end of surgery, two bolus doses of ketamine 20 mg was administered for the patient. Muscle relaxation was established with cisatracurium 4 mg intraoperatively and reversed with neostigmine and atropine at the end of surgery. The patient was mechanically ventilated with tidal volume of 400 ml and respiratory rate of 12/min. The fluids given to the patient in the operating room included isogroup partially cross-matched packed red blood cell 8 units, fresh frozen plasma 4 units, lactated Ringer's solution 2000 ml, hypertonic saline 5% 200 ml, normal saline 1000 ml, and mannitol 20% 150 ml. The patient was transported to the surgical intensive care unit (ICU), while intubated but with spontaneous breathing. He was not awake but showed motor response to painful stimulation.

The patient remained intubated in the ICU for 48 h under sedation with morphine sulfate and midazolam. Magnesium sulfate 1 g/h and maintenance dose of amiodarone were infused for the first 24 h postoperatively. During this period, respiratory support was performed with continuous positive airway pressure mode with pressure support 10 cm H_2O and positive end expiratory pressure equal to 5 cm H_2O. The patient tolerated this mode comfortably. Laboratory data in the first postoperative day showed increased creatinine (Cr = 1.6), hyperkalemia (K = 7.5), hypocalcemia (Ca = 6.7), hemoglobin = 10.6 mg/dl, platelet count = 108,000 and international normalized ratio equal to 1.9. Other laboratory examinations, including blood sugar, liver function tests, and other electrolytes were within normal range. The patient was given kay oxalate for his hyperkalemia. Serum creatinine decreased gradually, and electrolytes normalized in the following days without the need for dialysis.

The patient was extubated in the 3rd postoperative day when he became conscious. Analgesia with elastomeric infusion pump containing morphine sulfate was continued for the next 3 days and then changed to oral analgesic medications. Chest tube was removed in the 5th postoperative day. On the 8th postoperative day, he was discharged to home with good general condition without any neurologic sequel.

Discussion

The reported patient survived from 40 min of open-chest CPR without any neurologic sequel. The effective CPR along with protection against ischemia-reperfusion injury in the vital organs is the key factor in survival from prolonged cardiac arrest. Uninterrupted open-chest cardiac massage was performed for the reported patient. However, it is expectable to have some degrees of ischemia in vital organs after 40 min of resuscitation followed by reperfusion injury in heart, lung, brain, and kidneys. We applied multiple strategies to limit the deleterious effects of reperfusion and inflammation in this patient including administration of magnesium sulfate and mannitol, protective lung ventilation, and optimal postoperative pain control.

We administered magnesium sulfate 2 g after defibrillation for its reported neuroprotective[2,3] and cardioprotective properties.[4,5] Noteworthy, preliminary studies suggest that magnesium may have renoprotective effects.[6] We also used mannitol 30 g for renal protection. Its clinical benefit in ischemic conditions such as during cross-clamp of aorta in cardiac surgery has been approved,[7] but its effects following resuscitation is not fully investigated. It may also reduce brain edema and may improve cerebral perfusion in patients with mild brain damage following resuscitation.[8] However, its overall contribution to survival has not been sufficiently disclosed.

Our patient was an intravenous drug user experiencing a wide spectrum of substances. It is established that drug abuse has deleterious effects on several organs via direct toxic effects or triggering inflammatory process. However, it is not known whether exposure to chronic inflammation specifically drug abuse can reduce the harmful effects of an acute inflammation such as ischemia reperfusion injury. It seems reasonable to generalize the "pre-conditioning mechanism" to this area based on the positive results of earlier preliminary studies. A study showed that a pre- or a post-conditioning treatment with extremely low doses of tetrahydrocannabinol provides effective long-term cognitive neuroprotection.[9] Another laboratory study showed that in vivo administration of morphine 12 h prior to hypoxia/hypoglycemia can induce neuroprotective effects.[10] It has been suggested that morphine dependence protects the kidney against ischemia/reperfusion injury via opioid receptor-dependent pathways.[11] The role of opioids in different forms of preconditioning including ischemic and pharmacologic insults has been described.[12] Taken together, it seems reasonable to conclude that drug abusers may show different responses to ischemic conditions. This hypothesis and its clinical applications need to be validated in further investigations.

Another interesting point in the clinical scenario of this patient was the successful defibrillation with external paddles. Classically, defibrillation in open heart surgery is performed with small surgical paddles using 10-20 J of electricity. The impedance will change in thoracotomy. Thus, external defibrillation may result in myocardial stunning secondary to the delivery of high-energy shocks or conversely the applied energy may be ineffective. In the absence of small sterile "surgical" paddles, standard external paddles were placed on the chest wall, and defibrillation was successfully attempted with monophasic 200 J shock. This successful experience suggests using standard paddles for defibrillation in open cardiothoracic surgeries when surgical paddles are not available.

Conflict of Interests

Authors have no conflict of interests.

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How to cite this article: Mohseni M, Ziaeifard M, Abbasi Z. **Protection against ischemia-reperfusion injury in prolonged resuscitation: A case report and review of literature.** ARYA Atheroscler 2014; 10(4): 227-9.

Undiagnosed interrupted aortic arch in a 59-year-old male patient with severe aortic valve stenosis: A case report and literature review

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Case Report

Abstract

BACKGROUND: Interrupted aortic arch (IAA) is defined by a lack of the luminal continuity between the ascending and descending thoracic aorta. It is a rare, severe congenital heart defect which without surgery is associated with high mortality in the neonatal period. The aims of this study were to present a case with IAA who was alive until the age of 59 years without any surgical intervention and to review the literatures that have presented IAA cases.

CASE REPORT: The patient was admitted with respiratory distress and pulmonary edema. Echocardiography showed the sever stenosis in aortic valve and sever left ventricular dysfunction. Cardiac catheterization and angiography confirmed interrupted aorta (type A). The descending thoracic aorta was supplied by extensive collateral vessels from the vertebrobasilar system down to the posterior chest wall and the spine. Surgical correction including coronary artery bypass graft and aortic valve replacement and repair of interruption of the aorta was performed. Three weeks later the patient was died due to uncontrollable gastrointestinal bleeding and hospital acquired pneumonia. We described diagnosis and management of our case.

CONCLUSION: This case was very interesting for us, because the patient had not been diagnosed until the recent presentation. Similar cases with this diagnosis do not reach adulthood, but our patient was alive up to 59 years of age.

Keywords: Interrupted, Aorta, Aortic Valve Stenosis, Thoracic Aorta, Aortic Arch

Introduction

Interrupted aortic arch (IAA) is a rare, severe congenital heart defect defined as a complete loss of luminal and anatomic continuity between ascending and descending aorta,[1] representing approximately 1% of congenital heart disease.[2] It usually occurs in association with the nonrestrictive ventricular septal defect and ductus arteriosus or, less commonly, with a large aortopulmonary window or truncus arteriosus.[3] In the presence of two ventricles, varying degrees of left ventricular (LV) outflow tract obstruction is often observed.[4,5] It occurs in three per million live births.[6,7] IAA has been classified into three types (A, B, and C) based on the site of the aortic interruption. In the type A, interrupted left aortic arch, the arch interruption occurs distally to the origin of the left subclavian artery. In type B, interrupted left aortic arch, the interruption occurs distal to the origin of the left common carotid artery. In the type C, interrupted left aortic arch, the interruption occurs proximally to the origin of the left common carotid artery. Type B interruption

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accounts for about two-third of cases, type A occurs in about one-third of cases, and type C is presented in less than 1% of cases.[3]

The IAA is a congenital cardiopathy which has devastating consequences, with a 75% mortality rate at 10 days and 90% at 12 months of life.[6] In infants, its' clinical presentation often involves severe congestive heart failure and if left untreated, most affected infants die within some days. Lobato et al., in their study, reported that few cases were with IAA, which most of them need surgical replacement.[8]

In our case report, we want to introduce a 59-year-old man with undiagnosed interrupted aorta and how we managed him live up to 59 years without further surgical intervention.

Case Report

A 59-year-old man was presented in our hospital because of respiratory distress since 3 days before admission, which had been gradually sever. He was admitted with impression of pulmonary edema. He had a history of uncontrolled diabetes mellitus, systemic hypertension and hyperlipidemia.

On physical examination, he was blind, with blood pressure of 210/140 mmHg, pulse rate about 150 beat/min and respiratory rate about 40 cycle/min. The pulses were equal in upper limbs. Both femoral pulses were equal but weak. There were diffuse moist rhales in both lungs and cardiac examination systolic ejection sound and murmur was audible in the aortic area and with less severity in apex and lower left sternal border.

Electrocardiography showed sinus tachycardia with complete left bundle branch block. After several hours of aggressive medical treatment, the patient's condition became relatively stable.

Echocardiography showed sever aortic valve stenosis (mean pressure gradient = 60 mmHg) and sever LV systolic dysfunction (LV ejection fraction = 25%).

After initial stabilization with conservative treatment, coronary angiography was done and revealed three vessels coronary artery disease.

Cardiac catheterization from right femoral artery showed occlusion of the distal thoracic aorta to the left subclavian artery and angiography from right brachial artery proved interrupted aorta (type A). We were not able to pass through aortic valve to the left ventricle because of valvular stenosis, and it seems that left internal mammary artery plays role in collateralization but we did not engage through it (Figure 1).
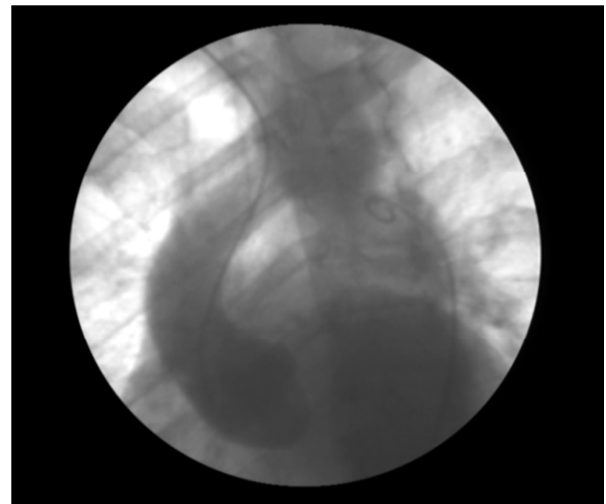

Figure 1. Angiography from right brachial artery proved interrupted aorta

The descending thoracic aorta was supplied by extensive collateral vessels from the vertebrobasilar system down to the posterior chest wall and the spine (Figure 2). Successful surgical correction including coronary artery bypass graft and aortic valve replacement and repair of interruption of the aorta was performed without any complication. Three weeks later the patient died due to gastrointestinal (GI) bleeding which was not controllable by aggressive treatment and hospital acquired pneumonia.
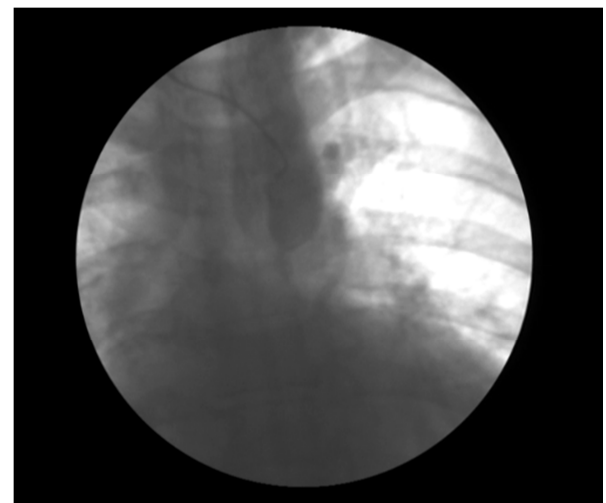

Figure 2. The descending thoracic aorta was supplied by extensive collateral vessels

Discussion

The IAA is a rare, severe congenital heart defect, which without surgery is associated with high mortality in the neonatal period;[8] but our case until age 59 without any surgery intervention was alive.

This disease displays the absence of communication between the two segments of the thoracic aorta and, consequently, of the blood flow; thus, most cases are expected to be fatal. In our case, cardiac catheterization showed occlusion of the distal thoracic aorta to the left subclavian artery (type A).[1]

The common characteristic among the survivors is the presence of an extensive collateral network, which is necessary for the maintenance of the distal flow and the consequent organ viability.[6]

In our case, the descending thoracic aorta was supplied by extensive collateral vessels from the vertebrobasilar system down to the posterior chest wall and the spine.

In this case, surgical correction was performed without any complication but the main reason for death of the patient was uncontrollable GI bleeding and hospital acquired pneumonia.

Conclusion

This case was very interesting for us, because the patient had not been diagnosed up to a recent presentation. Similar cases with this diagnosis do not reach adulthood, but our patient was alive up to his 60th decade.

Conflict of Interests

Authors have no conflict of interests.

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