Original Article

Serum Levels of Growth Differentiation Factor-15 as an Inflammatory Marker in Patients with Unstable Angina Pectoris

Fariba Raygan, MD[1], Aniseh Etminan, MD[2], Hanieh Mohammadi, MD[2], Hossein Akbari, MD[3], Hassan Nikoueinejad, MD[4*]

[1]*School of Medicine, Kashan University of Medical Sciences, Kashan, Iran.*
[2]*Students' Research Center, Kashan University of Medical Sciences, Kashan, Iran.*
[3]*School of Public Health, Kashan University of Medical Sciences, Kashan, Iran.*
[4]*Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.*

Received 26 June 2020; Accepted 19 November 2020

Abstract

Background: *Growth differentiation factor-15 (GDF-15), a member of transforming growth factors, is a stress-responsive marker whose levels may significantly increase in response to pathological stresses associated with inflammatory tissue injuries such as unstable angina pectoris (USAP). This study evaluated the diagnostic value of GDF-15 in patients with USAP.*

Methods: *The present cross-sectional study recruited 39 patients with USAP criteria and 30 patients with stable angina pectoris (SAP), referred to Shahid Beheshti Hospital, Kashan, Iran. All the patients with USAP had at least 1 coronary artery stenosis (>50%) in angiography. The control group comprised 42 healthy individuals. The serum levels of GDF-15 were measured in all the participants by ELISA. Also analyzed were the relationship between GDF-15 levels and thrombolysis in myocardial infarction (TIMI) and the Global Registry of Acute Coronary Events (GRACE) risk scores in the patients with USAP to determine the severity of the disease.*

Results: *The study population consisted of 111 subjects, 62 women and 49 men, divided into 3 groups of USAP (n=39, mean age=60.07±14.10 y), SAP (n=30, mean age=67.56±9.88 y), and control (n=42, mean age=61.21±7.76 y). The mean serum level of GDF-15 in the USAP group was significantly different from the other 2 groups (P<0.001), while no significant difference was observed in this regard between the SAP and control groups (P=0.797). No correlation was found between the mean GDF-15 serum level and the GRACE (P=0.816) and TIMI (P=0.359) risk scores in the USAP group.*

Conclusion: *The mean serum level of GDF-15 exhibited a rise in our patients with USAP. GDF-15 may be a diagnostic biomarker of USAP and its severity.*

J Teh Univ Heart Ctr 2021;16(1):15-19

This paper should be cited as: Raygan F, Etminan A, Mohammadi H, Akbari H, Nikoueinejad H. Serum Levels of Growth Differentiation Factor-15 as an Inflammatory Marker in Patients with Unstable Angina Pectoris. J Teh Univ Heart Ctr 2021;16(1):15-19.

Keywords: *Unstable angina; Stable angina; GDF-15; GRACE risks score; TIMI risk score*

Introduction

Acute coronary syndromes (ACSs) contain different clinical presentations compatible with myocardial ischemia and include unstable angina pectoris (USAP), non–ST-elevation myocardial infarction (NSTEMI), and ST-elevation

[*]**Corresponding Author:** ***Hassan Nikoueinejad,*** *Associate Professor of Immunology, Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Baqiyatallah Hospital, Mollasadra Ave, Vanak Sq, Tehran, Iran. 1435915371. Tel: +98 21 88621539. Fax: +98 21 81262073. E-mail: hnikuinejad@yahoo.com.*

myocardial infarction (STEMI).[1] ACSs constitute one of the most important health problems in that they affect about 1.5 million persons a year.[2] A timely diagnosis of ACSs significantly improves their clinical outcomes.[2] USAP is very common and serious. In the United States, it is responsible for more than 750 000 hospital admissions annually, with more than 70 000 patients developing myocardial infarction (MI) and some expiring suddenly.[3] USAP may occur at rest or with low workloads.[4] The disease procedure spreads from coronary vasospasm to thrombus formation and from no significant stenosis to severe triple-vessel disease.[5] How to screen high-risk patients due to ACSs is the key to the successful management of life-threatening coronary artery disease.[6] Typical electrocardiograms (ECGs) and cardiac necrosis biomarkers are helpful in the diagnosis of MI; however, the diagnosis of USAP remains clinical.[6] The traditional biomarkers that reveal myocardial necrosis represent the severe and late stages of ACSs and may not aid as diagnostic markers for all subtypes of ACSs.[6] Therefore, the introduction of novel biomarkers relating to the severity of coronary artery lesions seems necessary.[6] In USAP, small erosion or fissuring in atherosclerotic plaques may change their structure and cause a reduction in coronary blood flow, resulting in the exacerbation of the angina.[7] Inflammatory mediators are intimately associated with the cascade of events, leading to the initiation, development, and rupture of atherosclerotic plaques.[8]

Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor cytokine superfamily.[9] While GDF-15 is weakly expressed in most tissues under physiological situations,[10] its expression levels may significantly increase in any pathological stress associated with inflammation or tissue injury.[11, 12] In the heart of animal models, GDF-15 is induced in response to ischemia-reperfusion injury, pressure overload, and heart failure.[12, 13] It has been reported that the serum levels of GDF-15 are independently associated with the mortality and risk of recurrent MI.[14, 15]

Thrombolysis in myocardial infarction (TIMI) and the Global Registry of Acute Coronary Events (GRACE) risk scores are the most frequently used criteria[16] that help categorize patients' risk of ischemic events (MI and recurrent ischemia), bleeding, and death at initial evaluation of patients with USAP and NSTEMI.[17]

In this study, we aimed to evaluate the diagnostic value of GDF-15 in patients with USAP. We also evaluated the prognostic value of such serum markers in patients with USAP using the GRACE and TIMI risk scoring systems.

Methods

This cross-sectional study recruited 30 patients with SAP and 39 patients with USAP referred to the Emergency Department of Shahid Beheshti Hospital, affiliated with Kashan University of Medical Sciences, with chest pain and equivalent complaints and 42 healthy controls. The patients with USAP, evaluated by an emergency department physician and transferred to the cardiac care unit, had at least 1 coronary artery obstruction (>50%) in coronary artery angiography. The inclusion criterion was angina pectoris or equivalent ischemic discomfort with at least 1 of the following 3 features: 1) it occurs at rest or with minimal exertion and usually lasts for longer than 10 minutes, 2) it is severe and is of new onset (ie, within the prior 4–6 wk), and/or 3) it occurs with a crescendo pattern (ie, distinctly more severe, prolonged, or frequent than before). The patients with SAP were selected from those referred to the cardiology clinic. Healthy controls were volunteers who had no symptoms or history of heart disease. A history of chronic renal failure (glomerular filtration rate>20), active or previous cancers, recent infections or any inflammatory diseases, a history of MI, cirrhosis, and a history of addiction were considered the exclusion criteria. For each patient, the TIMI and GRACE risk scores were calculated using specific variables collected at admission (Table 1 and Table 2). The serum of all the subjects was separated from 3 mL of their venous blood samples through centrifugation at 4°C and then stored at −20°C until use. The serum levels of GDF-15 from all the contributors were determined by sandwich ELISA (Reddot, Biotech, Canada) in keeping with the company's instructions.

The statistical analyses were performed on SPSS, version 16.0. Descriptive data were expressed as the mean±the standard deviation and percentages. The Kolmogorov-Smirnov test was used to test for normal distributions among continuous variables. The categorical variables were compared by using the χ^2 test, while ANOVA and the t test were applied for the continuous variables. The Person correlation coefficient was utilized to determine the correlation between the continuous variables. A P value of less than 0.05 was considered statistically significant. A linear multiple regression analysis was performed to verify multivariate correlations between the GRACE and TIMI risk scores in the USAP group with the serum level of GDF-15 as a dependent variable. Receiver operating characteristic (ROC) curves were employed to relate the calculated serum levels of GDF-15 to the diagnosis of the disease. The area under the curve (AUC) was drawn upon as a measure of the predictive accuracy of the risk score. The cutoff points were identified with the ROC curves for GDF-15 to distinguish the study population as USAP and SAP groups.

Results

The study population consisted of 111 participants, divided into 3 groups of USAP (n=39), SAP (n=30), and control

(n=42). No difference was observed between the groups with regard to sex (P=0.497) and age distribution (P=0.013). For each patient, the TIMI and GRACE risk scores were calculated using specific variables collected at admission. The mean serum level of GDF-15 was significantly higher in the USAP group than in the other 2 groups (P<0.001) (Table 1). No difference was observed between the SAP group and the healthy control group concerning GDF-15 serum levels (P=0.733) (Table 1). The linear regression analysis, performed between the GRACE and TIMI risk scores and the mean GDF-15 serum level of the USAP group, revealed no correlation between the mean GDF-15 serum level and the GRACE risk score (P=0.816) (Table 2) (Figure 1). No correlation was also found between the mean GDF-15 serum level measured at baseline and the TIMI risk score (P=0.359) (Table 2 and Figure 2).

Table 1. Demographic and clinical characteristics of the patients and control groups*

	Unstable Angina	Stable angina	Control	P
Sex (F/M)	23/16	14/16	25/17	0.494
Age (y)				0.013
<59	20 (51.3%)	6 (20%)	20 (47.6%)	
60–69	9 (23.1%)	13 (43.3%)	16 (38.1%)	
≥70	10 (25.6%)	11 (36.7%)	6 (14.3%)	
Mean±SD	60.07±14.10	67.56±9.88	61.21±7.76	
GDF-15 (ng/mL)	2.30±0.76	0.89±0.48	0.85±0.39	<0.001

*Data are presented as mean±SD or n (%)
GDF-15, Growth differentiation factor-15

Table 2. Mean±standard deviation of the GRACE and TIMI scores and the Pearson correlation between the serum levels of GDF-15 and the severity of unstable angina pectoris

	N	Mean±SD	Std Error	GDF-15 Pearson Correlation	P
Age (y)					
GRACE					
<59	20	82.0±18.17	4.063	−0.336	0.148
60–69	9	106.11±12.13	4.046	0.571	0.108
≥70	10	135.70±18.34	5.80	−0.209	0.562
Mean±SD	39	101.33±28.08	4.497	0.006	0.816
TIMI					
<59	20	2.15±1.18	0.264	0.290	0.214
60–69	9	3.66±0.86	0.288	−0.756	0.018
≥70	10	3.60±1.17	0.371	0.289	0.418
Mean±SD	39	2.87±1.32	0.211	0.151	0.359

GDF-15, Growth differentiation factor-15; GRACE, Global Registry of Acute Coronary Events; TIMI, Thrombolysis in myocardial infarction

ROC curves were defined to investigate the diagnostic power of GDF-15 serum levels for the diagnosis of USAP and SAP (Figure 3 and Figure 4). The AUC of the ROC curves showed that the power of the mean GDF-15 serum level for the diagnosis of USAP was higher than that for SAP. The sensitivity and specificity of the mean GDF-15 serum level for the diagnosis of USAP were very high (sensitivity=97.4% and specificity=71%) (Table 3). The AUC value of the mean GDF-15 serum level for USAP diagnosis was 0.96. The best cutoff point of the mean GDF-15 serum level for the diagnosis of USAP was 1.11 (Table 3). However, the sensitivity and specificity of the mean GDF-15 serum level for the diagnosis of SAP were not as high as those for the diagnosis of USAP (sensitivity=70% and specificity=40.5%) (Table 3). The AUC value of the mean GDF-15 serum level for the diagnosis of SAP was 0521 (Table 3). The best cutoff point of the mean GDF-15 serum level for the diagnosis of SAP was 0.695.

Table 3. Accuracy of GDF-15 in the diagnosis of stable angina and unstable angina

	Stable Angina	Unstable Angina
Cutoff point	0.695	1.11
Sensitivity %	70	97.4
Specificity %	40.5	71.4
Positive predictive value %	45.7	76
Negative predictive value %	65.4	96.8
Accuracy %	0.521	0.96

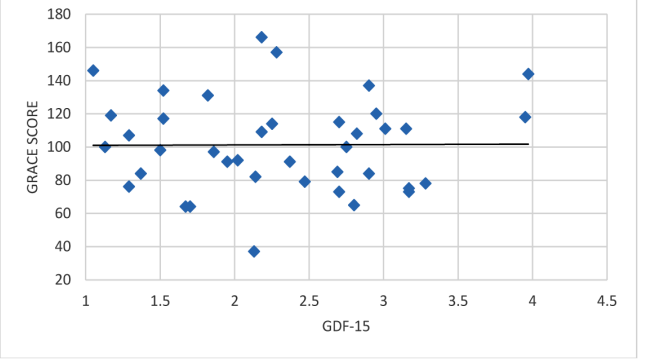

Figure 1. The image illustrates the correlation between the serum levels of GDF-15 and the GRACE risk score. A scatter plot of GDF-15 serum levels against the GRACE score shows no correlation between these variables (P=0.816, r=0.006).
GDF-15, Growth differentiation factor-15, GRACE, Global Registry of Acute Coronary Events

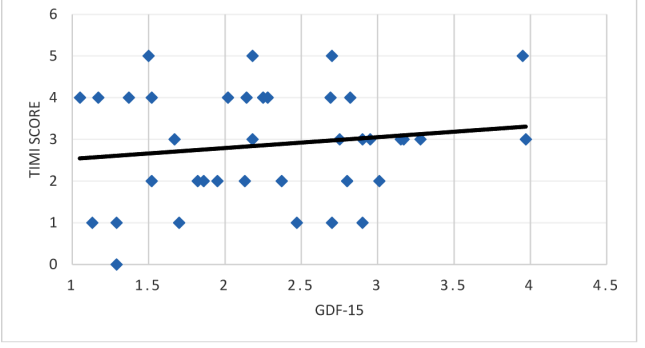

Figure 2. The image illustrates the correlation between the serum levels of GDF-15 and the TIMI risk score. A scatter plot of GDF-15 serum levels against the TIMI score shows a close correlation between these variables (P=0.359, r=0.151).

GDF-15, Growth differentiation factor-15, GRACE, Global Registry of Acute Coronary Events; TIMI, Thrombolysis in myocardial infarction

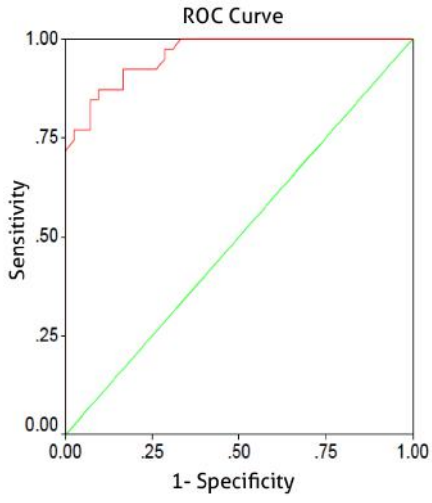

Figure 3. The image demonstrates the sensitivity and specificity of GDF-15 (ng/mL) for the diagnosis of USAP. The diagram shows that serum levels of GDF-15 could predict USAP with a sensitivity of 97.4% and a specificity of 71.4% using a cutoff value of 1.11 (AUC=1.96, P<0.001, 95%CI=0.925–0.995).
GDF-15, Growth differentiation factor-15; USAP, Unstable angina pectoris; AUC, Area under the curve

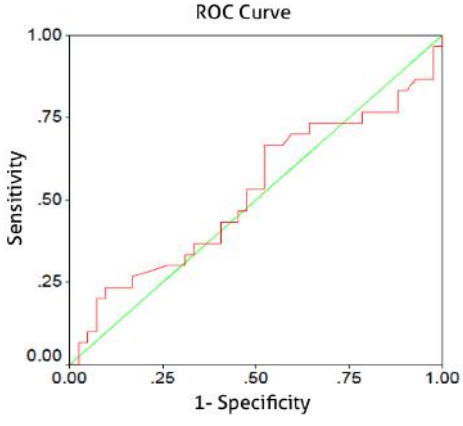

Figure 4. The image illustrates the sensitivity and specificity of GDF-15 (ng/mL) for the diagnosis of SAP. The diagram shows that serum levels of GDF-15 could predict SAP with a sensitivity of 70% and a specificity of 40.5% using a cutoff value of 0.695 (AUC=0.521, P=0.758, 95%CI=0.381–0.661).
GDF-15, Growth differentiation factor-15; SAP, Stable angina pectoris; AUC, Area under the curve

Discussion

USAP is a clinically diagnosed syndrome characterized by the new onset of angina at minimal exertions or at rest and/or any prolonged anginal pain at rest without infarction or crescendo angina.[18] In USAP, inflammatory-cell infiltration is commonly found in most atherosclerotic plaques of endarterectomy specimens.[19, 20]

The conventional biomarkers that indicate myocardial necrosis may not be used as indicators of all forms of ACSs. 6 Search for novel biomarkers that are directly linked to the severity of coronary artery lesions is warranted.[21] GDF-15 is secreted from human macrophages activated by pro-inflammatory cytokines,[22] endothelial cells,[23] vascular smooth muscle cells,[24] and adipocytes.[25] It has also been found to be secreted from murine myocardial tissue in response to ischemia and reperfusion and infarcted human myocardium.[12, 13] GDF-15 is also expressed in the myocardium of patients with acute MI.[12] This biomarker adds prognostic information to clinical risk indicators and established biomarkers in NSTEMI-ACSs and heart failure, indicating that GDF-15 may provide an insight into a distinct pathophysiological axis.[14, 26] Reasonably, inflammation comprises some part of such an axis. Supporting an association between GDF-15 and inflammation, Kempf et al.[27] showed that GDF-15 levels were significantly related to C-reactive protein in patients suffering from ACSs. Patients with no apparent cardiovascular diseases or other inflammatory illnesses exhibit low levels of GDF-15 when compared with patients with NSTEMI-ACSs or chronic heart failure.[27]

Chiming in with our findings, a few other studies have explored the diagnostic and prognostic values of GDF-15 in coronary artery disease states. Khan et al.[28] introduced GDF-15 as a prognostic marker of death and heart failure in patients with MI. Kempf et al.[9] identified GDF-15 as a promising new biomarker for the risk stratification of patients with coronary heart disease. Wollert et al.[14] concluded that GDF-15 was a new biomarker of the risk of death in patients with NSTEMI-ACSs in that it conferred prognostic information beyond that provided by established clinical and biochemical markers. They also reported that their patients with NSTEMI-ACSs displayed significantly higher GDF-15 levels than their healthy controls. In line with these studies, we showed that GDF-15 might be a diagnostic and prognostic marker of USAP. Using ROC curves to evaluate the diagnostic specificity and sensitivity of GDF-15 serum levels for USAP, we found that such sensitivity and specificity appear to be very high. Considering that the negative predictive value of GDF-15 serum levels is 96.85, we may conclude that patients with GDF-15 serum levels less than 1.11 do suffer from USAP, with a probability of 96.8%.

The results of the present study should be interpreted in light of some limitations. First, we did not take longitudinal follow-up samples to evaluate GDF-15 changes over time; such a limitation allowed for just a cross-sectional analysis. Second, we cannot comment on the diagnostic and prognostic accuracy of GDF-15 among patients with the exclusion criteria of our investigation such as USAP patients with renal failure.

Conclusion

The results of the current study demonstrated an elevation

in the mean serum level of GDF-15 in patients with unstable angina pectoris. GDF-15 may be a diagnostic biomarker of unstable angina pectoris and its severity in the early stages of the disease.

Acknowledgments

This study was derived from studies related to a research supported by the Deputyship of Research, Kashan University of Medical Sciences (Grant No 3792).

References

1. Baumann AAW, Mishra A, Worthley MI, Nelson AJ, Psaltis PJ. Management of multivessel coronary artery disease in patients with non-ST-elevation myocardial infarction: a complex path to precision medicine. Ther Adv Chronic Dis 2020;11:2040622320938527.
2. Aarts GWA, Mol JQ, Camaro C, Lemkes J, van Royen N, Damman P. Recent developments in diagnosis and risk stratification of non-ST-elevation acute coronary syndrome. Neth Heart J 2020;28(Suppl 1):88-92.
3. Graham D. Detailed diagnoses and procedures for patients discharged from short-stay hospitals. Vital Health Stat 13 1988;95:1-400.
4. Amane HS, Burte NP. A comparative study of dalteparin and unfractionated heparin in patients with unstable angina pectoris. Indian J Pharmacol 2011;43:703-706.
5. Théroux P, Fuster V. Acute coronary syndromes: unstable angina and non-Q-wave myocardial infarction. Circulation 1998;97:1195-1206.
6. Zheng W, Lai Y, Jin P, Gu W, Zhou Q, Wu X. Association of circulating IGFBP1 level with the severity of coronary artery lesions in patients with unstable angina. Dis Markers 2017;2017:1917291.
7. Jinnouchi H, Sato Y, Sakamoto A, Cornelissen A, Mori M, Kawakami R, Gadhoke NV, Kolodgie FD, Virmani R, Finn AV. Calcium deposition within coronary atherosclerotic lesion: Implications for plaque stability. Atherosclerosis 2020;306:85-95.
8. George M, Shanmugam E, Srivatsan V, Vasanth K, Ramraj B, Rajaram M, Jena A, Sridhar A, Chaudhury M, Kaliappan I. Value of pentraxin-3 and galectin-3 in acute coronary syndrome: a short-term prospective cohort study. Ther Adv Cardiovasc Dis 2015;9:275-284.
9. Kempf T, Sinning JM, Quint A, Bickel C, Sinning C, Wild PS, Schnabel R, Lubos E, Rupprecht HJ, Münzel T, Drexler H, Blankenberg S, Wollert KC. Growth-differentiation factor-15 for risk stratification in patients with stable and unstable coronary heart disease: results from the AtheroGene study. Circ Cardiovasc Genet 2009;2:286-292.
10. Wischhusen J, Melero I, Fridman WH. Growth/differentiation factor-15 (GDF-15): from biomarker to novel targetable immune checkpoint. Front Immunol 2020;11:951.
11. Hsiao EC, Koniaris LG, Zimmers-Koniaris T, Sebald SM, Huynh TV, Lee SJ. Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. Mol Cell Biol 2000;20:3742-3751.
12. Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, Heineke J, Kotlarz D, Xu J, Molkentin JD, Niessen HW, Drexler H, Wollert KC. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. Circ Res 2006;98:351-360.
13. Xu J, Kimball TR, Lorenz JN, Brown DA, Bauskin AR, Klevitsky R, Hewett TE, Breit SN, Molkentin JD. GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. Circ Res 2006;98:342-350.
14. Wollert KC, Kempf T, Peter T, Olofsson S, James S, Johnston N, Lindahl B, Horn-Wichmann R, Brabant G, Simoons ML, Armstrong PW, Califf RM, Drexler H, Wallentin L. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. Circulation 2007;115:962-971.
15. Wollert KC, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Allhoff T, Peter T, Siegbahn A, Venge P, Drexler H, Wallentin L. Growth differentiation factor 15 for risk stratification and selection of an invasive treatment strategy in non ST-elevation acute coronary syndrome. Circulation 2007;116:1540-1548.
16. Silva FM, Pesaro AE, Franken M, Wajngarten M. Acute management of unstable angina and non-ST segment elevation myocardial infarction. Einstein (Sao Paulo) 2015;13:454-461.
17. Antman EM, Cohen M, Bernink PJ, McCabe CH, Horacek T, Papuchis G, Mautner B, Corbalan R, Radley D, Braunwald E. The TIMI risk score for unstable angina/non-ST elevation MI: a method for prognostication and therapeutic decision making. JAMA 2000;284:835-842.
18. Cagle SD, Jr, Cooperstein N. Coronary artery disease: diagnosis and management. Prim Care 2018;45:45-61.
19. Coveney S, McCabe JJ, Murphy S, O'Donnell M, Kelly PJ. Anti-inflammatory therapy for preventing stroke and other vascular events after ischaemic stroke or transient ischaemic attack. Cochrane Database Syst Rev 2020;5:CD012825.
20. Budzianowski J, Pieszko K, Burchardt P, Rzeźniczak J, Hiczkiewicz J. The Role of Hematological Indices in Patients with Acute Coronary Syndrome. Dis Markers 2017;2017:3041565.
21. Centurión OA. Serum biomarkers and source of inflammation in acute coronary syndromes and percutaneous coronary interventions. Cardiovasc Revasc Med 2016;17:119-128.
22. Bauskin AR, Brown DA, Kuffner T, Johnen H, Luo XW, Hunter M, Breit SN. Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. Cancer Res 2006;66:4983-4986.
23. Secchiero P, Corallini F, Gonelli A, Dell'Eva R, Vitale M, Capitani S, Albini A, Zauli G. Antiangiogenic activity of the MDM2 antagonist nutlin-3. Circ Res 2007;100:61-69.
24. Bermúdez B, López S, Pacheco YM, Villar J, Muriana FJ, Hoheisel JD, Bauer A, Abia R. Influence of postprandial triglyceride-rich lipoproteins on lipid-mediated gene expression in smooth muscle cells of the human coronary artery. Cardiovasc Res 2008;79:294-303.
25. Ding Q, Mracek T, Gonzalez-Muniesa P, Kos K, Wilding J, Trayhurn P, Bing C. Identification of macrophage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes. Endocrinology 2009;150:1688-1696.
26. Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. J Am Coll Cardiol 2007;50:1054-1060.
27. Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G, Drexler H, Johnston N, Wallentin L, Wollert KC. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. Clin Chem 2007;53:284-291.
28. Khan SQ, Ng K, Dhillon O, Kelly D, Quinn P, Squire IB, Davies JE, Ng LL. Growth differentiation factor-15 as a prognostic marker in patients with acute myocardial infarction. Eur Heart J 2009;30:1057-1065.