Author's response to reviews

Title: Implication of Plasma Intermediin Levels In Patients Who Underwent First-time Diagnostic Coronary Angiography: a single centre, cross-sectional study

Authors:

Aylin Hatice Yamac (aylin-yamac@yandex.com)
Ahmet Bacaksiz (ahmetbacaxiz@yandex.com)
Ziya Ismailoglu (ziyaismail@yahoo.com)
Sitki Kucukbuzcu (s_kucukbuzcu@hotmail.com)
Emrah Sevgili (sevgiliemrah@yahoo.com)
Emin Asoglu (asemctff@hotmail.com)
Muharrem Nasifov (drmeherrem@yahoo.com)
Parviz Jaferov (doctorperviz@gmail.com)
Ercan Erdogan (drercanerdogan@gmail.com)
Omer Goktekin (ogoktekin@gmail.com)

Version: 3
Date: 24 October 2014

Author's response to reviews: see over
Dear Editor,

The authors have revised the manuscript entitled "Implication of Plasma Intermedin Levels In Patients Who Underwent First-time Diagnostic Coronary Angiography" and have responded to the reviewers’ comments. A point-by-point response to reviewers have provided. We have highlighted the changes in the manuscript by using colored text (for excluded parts in red and for added parts in yellow colors).

We are looking forward to your favorable response in your early convenience.

Yours sincerely,

Aylin Yamac, M.D.
Responses to Reviewers’ Comments:

Reviewer 1:

Comment: "I haven't any major revision but only a suggestion: what about changing the title in: "Implication of Plasma Intermedin Levels In Patients Who Underwent First-time Diagnostic Coronary Angiography: a single centre, cross-sectional study"?"

Reply: We changed the title as "Implication of Plasma Intermedin Levels In Patients Who Underwent First-time Diagnostic Coronary Angiography: a single centre, cross-sectional study" according to the reviewer’s suggestions.

Reviewer 2:

Comment: "as the Intermedin is expressed primarily in the pituitary and gastrointestinal tracts, you should have previously screened the patients for neurological and gastro-intestinal disease, as it could influence the results."

Reply: Although the primary source of intermedin (IMD) is not exactly known but pituitary glands are accepted as the main source as commented by the reviewer, transcriptional expression and immunohistochemical investigation in rodents and humans revealed high levels of IMD in the kidney, muscularis mucosa of the stomach and jejunum, pancreas, spleen, lung, hypothalamus, skin, submaxillary gland, thymus, ovaries and testis (Roh J, Chang CL, Bhalla A, Klein C, Hsu SY. Intermedin is a calcitonin/calcitonin gene-related peptide family peptide acting through the calcitonin receptor-like receptor/receptor activity-modifying protein receptor complexes. J Biol Chem 2004; 279:7264-74). In normal mature mouse heart, IMD expression is at low levels from endothelial cells of the coronary artery and veins and cardiomyocytes (Kobayashi Y, Liu YJ, Gonda T, Takei Y. Coronary vasodilatory response to a novel peptide, adrenomedullin 2. Clin Exp Pharmacol Physiol 2004; 31 Suppl 2:S49-50). Similarly, lower levels of IMD in healthy human myocardial cells are elevated
under stress. Up to date, large amount of data showed that IMD expression is augmented in various vascular diseases such as atherosclerosis, coronary artery disease (CAD), essential and pulmonary arterial hypertension (Ni X, Zhang J, Tang C, Qi Y. Intermedin/adrenomedullin2: an autocrine/paracrine factor in vascular homeostasis and disease. Sci China Life Sci 2014; 57:781-9).

We agree with the reviewer that pathologies involving gastrointestinal tract or pituitary glands could change serum IMD levels. None of the patients in our study had known or overt GI system/pituitary gland disease. We did not screen patients for neurological and gastrointestinal diseases due to medico-economic constraints and reimbursement problems in our country. Also, none of the previous studies in the literature (Qin YW, Teng X, He JQ, Du J, Tang CS, Qi YF. Increased plasma levels of intermedin and brain natriuretic peptide associated with severity of coronary stenosis in acute coronary syndrome. Peptides 2013; 42:84-8) (Lv Z, Wu K, Chen X, Zhang X, Hong B. Plasma intermedin levels in patients with acute myocardial infarction. Peptides 2013; 43:121-5) (Tang B, Zhong Z, Shen HW, Wu HP, Xiang P, Hu B. Intermedin as a prognostic factor for major adverse cardiovascular events in patients with ST-segment elevation acute myocardial infarction. Peptides 2014; 58:98-102) did not take into account the patients' accompanying GI system or pituitary diseases, so we assume that elevated levels of IMD in our patients resulted from CAD.

We included this issue to the last paragraph of Discussion as "Other pathologies that could potentially elevate serum IMD levels such as gastrointestinal, pituitary, other organ or body systems did not take into account."

*Comment: "As also other clinical conditions besides CAD could increase the IMD levels, please provide echocardiographic data and the differences among the groups in left ventricular hypertrophy, left ventricular ejection fraction, chronic obstructive
pulmonary disease, glomerular filtration rate, peripheral vascular disease. Please add to the laboratory features the C-reactive protein, as a marker of inflammatory status. Regarding the cut off values of IMD predicting CAD, I suggest to explicit their accuracy in the results section."

Reply: We performed transthoracic echocardiography (TTE) to all study patients in order to rule out heart failure as we stated at the Materials and Methods section as "Exclusion criteria included histories of recent myocardial infarction (MI), unstable angina pectoris (UAP), heart failure (systolic and/or diastolic heart failure was excluded with the use of transthoracic echocardiography, left ventricular ejection fraction <40% was accepted as systolic heart failure), moderate to severe heart valve disease, malignancies, major trauma or surgery in the previous six months, renal insufficiency, acute or chronic infectious disease, any kind of immune-mediated disease, since all these conditions may affect plasma IMD levels". None of the patients had systolic/diastolic heart failure or hypertrophic cardiomyopathy. It could be reasonable to assess the effect of echocardiographical measurements such as left ventricular ejection fraction (EF) on serum IMD levels but most of the patients had normal or near normal EF, so we did not attach TTE parameters to the manuscript. Also, the scope of this article is to evaluate serum IMD levels as a predictor of CAD, adding TTE derived measures could digress the subject.

Patients with severe chronic obstructive pulmonary disease were not included into the study and we added this explanation to the Materials and Methods section of the manuscript.

Patients with renal insufficiency were excluded from the study as expressed in the Materials and Methods section. Although we did not calculate glomerular filtration rate of
each patient, mean creatinine levels of the study groups were normal (0.7 ± 0.1, 0.8 ± 0.2 and 0.8 ± 0.2 mg/dL in group 1, 2 and 3 consecutively).

None of the patients had known or overt peripheral artery disease (PAH). We did not screen patients for PAH. We expressed this issue at the last paragraph of the Discussion as "Also, patients were not investigated for other potential sites of atherosclerosis, such as carotid artery, thoraco-abdominal aorta, renal arteries, and peripheral vascular bed."

We did not measure C-reactive protein levels of the patients due limited budget of the study and expressed this issue at the last paragraph of the Discussion as "The main limitation of our study was that other markers of atherogenesis such as hsCRP, chemotactic molecules, interleukin-6 and growth differentiation factor-15 were not determined."

**Comment:** "The discussion section is adequately supported by the data and balanced quite well. Please provide any potential clinical implications of your findings. Moreover, Authors suggest that plasma IMD levels correlate with Gensini and SYNTAX scores, even though the correlation between IMD-Syntax score is quite poor (r=0.29). How do you explain it? Please explicit in the Discussion section."

**Reply:** Possible potential implications of this study could be regarded as follows:

- measurement of serum IMD levels could support the clinician about the presence and severity of CAD before coronary angiography.

- unnecessary coronary angiographies could be prevented by measuring serum IMD levels.
- as a biomarker of atherosclerotic progression, patients with elevated serum IMD levels could be treated more intensely, for example high dose statins, renin/angiotensin system blockers and antiplatelets. Also, close medical monitoring for the patients with elevated serum IMD levels could be reasonable to early detection and treatment of atherosclerotic involvement.

Although the correlation between serum IMD levels and angiographic indices such as vessel and Gensini score was strong (rs = 0.710 and rs = 0.742, respectively), a diminution was stood out in correlation coefficient for SYNTAX score (rs = 0.296). The SYNTAX score is a relatively new index compared to the others and primary focus on coronary anatomy, which includes defining the coronary dominance and characterizing the presence and features of chronic total occlusions, trifurcations, bifurcations, aorto-ostial lesions, severe tortuosity, long lesions, heavy calcification, thrombus and/or diffuse disease. The SYNTAX score originated from the landmark Synergy between PCI with Taxus and Cardiac Surgery (SYNTAX) trial in 2009 which sought to establish whether coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI) was the standard of care for patients with three-vessel or left main coronary artery disease. Although the score is used primarily to risk-stratify these complex patients, the use of the score for appropriate risk stratification in order to determine the optimal revascularization strategy in all CAD types are very popular nowadays. In our study, the mean SYNTAX score was very low (15.1 ± 7.8) due to a few number of patients with complex disease (for example, 2 patients with LMCA stenosis). We assume that poor correlation between SYNTAX score and serum IMD levels was resulted from relatively low SYNTAX scores and limited number of patients with high (>22) SYNTAX score in our study.

Comment: "The writing is acceptable, however the syntax need to be generally improved:}
for example change the second sentences of the first paragraph in the abstract

section in “Elevated plasma IMD levels have been demonstrated to correlate with the severity of coronary stenosis in patients with acute coronary syndromes. So far, no human study has examined the role of IMD in stable patients who underwent diagnostic coronary angiography””

**Reply:** We changed the Abstract according to the suggestions of the reviewer.