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Abstract

Aim: Prolidase is an enzyme, which plays a role in the formation of a new matrix, collagen metabolism and cell development. It is known that the most important mechanism underlying in-stent restenosis is neointimal hyperplasia. Neointimal hyperplasia is associated with collagen synthesis and matrix proteins. The objective of our study was to reveal the relationship between serum prolidase levels and in-stent restenosis.

Material and Methods: This study included a total of 70 patients who were identified to be at a moderate and high risk as a result of clinical or non-invasive tests in the cardiology and emergency clinics of the Abant Izzet Baysal University training and Research Hospital and who underwent angiography. In-stent restenosis was identified in 40 patients. In the remaining 30 patients, there was no angiographically determined critical lesion. Serum prolidase levels were measured in all patients.

Results: The mean serum level of prolidase was found to be statistically significantly higher in the in-stent restenosis group compared to the restenosis-free group (p=0.02). The mean serum level of prolidase level was significantly higher in smokers compared to the non-smoker patients (p=0.04). It was observed that serum prolidase levels statistically significantly increased proportionally to the in-stent restenosis percentage (p=0.04).

Discussion: The results of this study indicate that prolidase enzyme levels may enable timely and correct assessment of in-stent restenosis, and may contribute to the decision for changing the treatment or timing to increase the intensity of the treatment in patients undergoing percutaneous coronary intervention (PCI) with coronary stenting.

Keywords
Percutaneous Intervention, Coronary Stent, In-Stent Restenosis, Prolidase

DOI: 10.4328/ACAM.20689    Received: 2021-05-05    Accepted: 2021-07-17    Ann Clin Anal Med 2021;12(11):1198-1202
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Introduction
In the world, cardiovascular diseases are the most frequent cause of mortality [1]. Despite novel modern treatment methods and the use of complex interventional and surgical techniques, mortality rates from these diseases remain high [2]. CVDs involve coronary arterial diseases (CAD), peripheral vascular diseases, hypertension (HT), cerebrovascular diseases, congestive heart failure (CHF), and congenital and valvular heart diseases; whereas, CADs include angina, myocardial infarction (MI), coronary failure, and coronary death [3]. The known most important risk factors of CAD are age, diabetes mellitus (DM), HT, smoking, dyslipidemia, obesity and sedentary lifestyle [4]. CADs more than 1.2 million myocardial infarction (MI) cases and more than 500,000 deaths every year in the USA [5].

In the treatment of CAD, the most commonly used interventional techniques include percutaneous transluminal coronary angioplasty (PTCA), coronary stent implantation and some therapeutic coronary devices. These methods are collected under the umbrella of percutaneous coronary intervention (PCI) [6]. Today, approximately 1,000,000 PCI procedures are performed annually in the USA [7].

A coronary stent implantation is now more widely used since it leads to fewer complications and provides better long-term clinical outcomes compared to PTCA. One of the most important problems encountered following coronary stent implantation is restenosis of the coronary arterial stent. Coronary artery stent restenosis can be defined as a decrease in the artery diameter caused by the damage occurring during coronary revascularization and the resultant negative response against this condition [8].

Coronary artery restenosis often occurs in the form of in-stent restenosis (ISR). ISR has always been considered the "enemy" for interventional cardiologists, thus many technical advancements (bare metal, drug-eluting stents, drug-coated balloons) aimed at preventing ISR [9]. ISR has been shown to be an independent predictor for mortality during follow-up together with the other clinical risk factors including age, sex, DM, smoking, previous by-pass operation, and left ventricular ejection fraction [10]. Today, the rate of ISR may be as high as 60% to 80%. This rate may rise to 70% in patients with risk factors such as diabetes mellitus [11]. Coronary stent implantation has decreased the incidence of ISR compared to PTCA. Today, coronary stent implantation is the most frequently used method in the treatment of obstructive CAD to reduce ISR and is a better option than surgical revascularization. Despite advancements in medical and technological fields, ISR is a still not fully resolved problem. Smooth muscle cell proliferation and neointimal hyperplasia are the most important causes of ISR. This study was planned considering that serum prolidase enzyme, which plays a role in collagen formation, may be effective in ISR as well.

Prolidase is an enzyme that breaks peptide bonds in proline-containing dipeptides (X-Pro) and is found in many tissues such as bone, connective tissue, kidney, heart, intestinal mucosa, liver, brain, uterus, thymus, erythrocytes, leucocytes, fibroblasts and plasma. It contributes to the formation of new matrix and cellular growth in collagen metabolism. Prolidase plays a role in the catabolism of proteins containing procollagen, collagen, proline and hydroxyproline inside the cell [12]. Proline and hydroxyproline amino acids, released by the prolidase enzyme, make up about 25% of the collagen tissue and help maintain the continuity of connective tissue. The increased destruction of collagen causes an increase in serum prolidase activity [13]. In a study about prolidase, it was aimed to determine the relationship between serum prolidase activity and ischemia time in different ischemia types. In conclusion, it was found that serum prolidase enzyme activity may be an important biomarker in predicting ischemia time [14].

To our knowledge, so far there have been no studies investigating the value of serum prolidase in patients with stent restenosis. Therefore, the objective of this study is to reveal the relationship between ISR and serum prolidase levels.

Material and Methods
This study included a total of 70 patients who were identified to be at a moderate and high risk as a result of clinical or non-invasive tests in the cardiology and emergency clinics of the Abant Izzet Baysal University training and Research Hospital, and who underwent angiography between 2013 through 2014. Forty patients with in-stent restenosis were assigned to the study group and the remaining 30 patients without ISR to the control group, and the results were compared between both groups.

Before the beginning of the study, the necessary approval was received from the local ethics committee of our hospital dated 23/12/2013 and 2013/08-249 numbered decision, in accordance with the Patient Rights Regulation and ethical principles. This study was supported by the Abant Izzet Baysal University, Scientific Research Projects Unit with the Project No: BAP – 2014.08.31.731. All participants signed written informed consent forms.

Patients with acute coronary syndrome, serious valvular diseases, severe coronary artery disease that may affect serum prolidase level, collagen tissue disease, chronic kidney and liver failure and rheumatological disease were excluded from the study.

Patients’ age, gender, presence of DM, HT, cholesterol levels, smoking status, history of PCI, ejection fraction (%EF), biochemical and hematologic parameters, drugs used, angiography outcomes, ISR stenosis rates, stent types (bare-metal stent/drug-eluting stent) and serum prolidase levels were investigated in details and recorded.

All angiography and PCI procedures were performed by cardiologists experienced in interventional procedures using the Siemens Axiom Artis angiography device with standard methods. A narrowing of ≥50% in the lumen diameter in the stent area was considered restenosis. A venous blood sample of 8 cc was drawn to a biochemical tube, centrifuged at 4000 rpm for 10 minutes, and the obtained serum sample was stored at -72oC until the analysis. Serum prolidase level was then studied in this sample in the laboratory of AIBU Medical Faculty Biochemistry Department. Measurements were done with the ELISA method using the Cusabio kit.

Statistical Analysis:
Data obtained in this study were statistically analyzed using SPSS (15.0, Inc, Chicago, IL, USA) statistical software.
Categorical variables were expressed as frequency and percentage. Continuous variables were expressed as mean ± standard deviation or median values. The Student’s t-test was used to compare continuous variables between the groups, while the Chi-square test was used to compared categorical variables. To calculate the correlations between continuous variables, Pearson’s analysis was used in parametric data and Spearman’s method in non-parametric data. Linear regression analysis was performed to determined independent predictors of stent restenosis. P values <0.05 were considered statistically significant.

Results
A total of 70 patients who had stents in their coronary arteries within the last year and underwent coronary angiography due to any indication other than acute coronary syndrome were included in this study. ISR was found in 40 patients. The mean age was 63.3±10.3 years in the patient and 61.7±11.1 years in the control group (p=0.5). In the patient group, 9 patients were female and 31 were male, whereas in the control group, 11 were female and 19 male (p=0.19). The incidence of ISR was significantly higher in smokers compared to nonsmoker patients (Figure 1) (p=0.04). No statistically significant difference was found between ISR and control groups in terms of DM and HT (for all; p>0.05).

Again, no statistically significant difference was found between the ISR and control groups in age, echocardiographic parameters (left ventricular diastolic diameter, left ventricular systolic diameter, ejection fraction), urea, creatinine, hemoglobin, MCV, WBC, PLT, LDL, and NON-HDL cholesterol levels (for all; p>0.05).

Table 1 shows demographic features and Table 2 echocardiographic and laboratory parameters in patients with and without ISR.

When the drugs used were evaluated between the groups, no statistically significant difference was found between the ISR and control groups in terms of using beta blockers, statins, ACE inhibitors and/or angiotensin receptor blockers (ARB) (for all; p>0.05).

There was no significant difference between the ISR and control groups in terms of stent types and coronary arteries where the stent was inserted (for all; p>0.05) (Table 3). Coronary arteries with ISR are seen in Figure 2.

In the comparison between the increase of restenotic stent rate and serum levels of prolidase, it was observed that serum prolidase level increased as the rate of restenotic stents increased, and this relationship was statistically significant (p=0.04).

The GENSINI angiographic score was statistically significantly higher in the ISR group compared to the control group (35.7±26.8 vs 19.8±17) (p=0.002).

The serum level of prolidase was found to be statistically significantly higher in the ISR group than in the control group (993.18 ± 609.99 mU/mL vs 468.53 ± 258.92 mU/mL) (p=0.02).

A comparison of serum prolidase levels between ISR and control groups is shown in Figure 3.

In the multivariate linear regression analysis, independent predictors of stent restenosis were determined as prolidase, age, LDL and Gensini score. When correlations between the parameters were evaluated, there was a moderate correlation between prolidase level and restenotic stent rate according to Pearson’s correlation analysis (r: 0.44, p: 0.03). In the linear regression analysis, including serum prolidase level, age, LDL and Gensini’s score, serum prolidase level (β: 0.45, p: 0.02) and Gensini’s score (β: 0.56, p: 0.02) were found to be independent predictors of in-stent restenosis.
Table 1. Demographic features of ISR and control groups

<table>
<thead>
<tr>
<th></th>
<th>ISR</th>
<th>Control</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63.3±10.3</td>
<td>61.7±11.1</td>
<td>70</td>
<td>0.50</td>
</tr>
<tr>
<td>Female</td>
<td>9 (22%)</td>
<td>11 (27%)</td>
<td>20</td>
<td>0.19</td>
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<tr>
<td>Male</td>
<td>31 (78%)</td>
<td>19 (63%)</td>
<td>50</td>
<td>0.19</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28 (70%)</td>
<td>16 (53%)</td>
<td>44</td>
<td>0.15</td>
</tr>
<tr>
<td>No</td>
<td>12 (30%)</td>
<td>14 (47%)</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (32%)</td>
<td>12 (40%)</td>
<td>25</td>
<td>0.52</td>
</tr>
<tr>
<td>No</td>
<td>27 (68%)</td>
<td>18 (60%)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (25%)</td>
<td>2 (7%)</td>
<td>12</td>
<td>0.04</td>
</tr>
<tr>
<td>No</td>
<td>30 (75%)</td>
<td>28 (93%)</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test.

Table 2. Echocardiographic and laboratory parameters of ISR and control groups

<table>
<thead>
<tr>
<th></th>
<th>ISR</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDD (mm)</td>
<td>48±5.9</td>
<td>49.9±6.2</td>
<td>0.21</td>
</tr>
<tr>
<td>LVSD (mm)</td>
<td>32±7.2</td>
<td>33.3±7.6</td>
<td>0.43</td>
</tr>
<tr>
<td>EF (%)</td>
<td>51.9±10.3</td>
<td>53.2±12.7</td>
<td>0.63</td>
</tr>
<tr>
<td>UREA (mg/dL)</td>
<td>349.8±10.4</td>
<td>35.9±13.2</td>
<td>0.69</td>
</tr>
<tr>
<td>CREATININE (mg/dL)</td>
<td>0.96±0.18</td>
<td>1.07±0.87</td>
<td>0.46</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>13.7±1.54</td>
<td>13.8±1.91</td>
<td>0.69</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>85.7±4.77</td>
<td>85.1±12.3</td>
<td>0.24</td>
</tr>
<tr>
<td>WBC (K/uL)</td>
<td>7.27±2.27</td>
<td>7.89±2.34</td>
<td>0.23</td>
</tr>
<tr>
<td>PLT (K/uL)</td>
<td>231±4.61</td>
<td>246±65.5</td>
<td>0.24</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>101.8±35.5</td>
<td>101.8±38.2</td>
<td>0.99</td>
</tr>
<tr>
<td>NON HDL (mg/dL)</td>
<td>153.2±42.7</td>
<td>153.5±40.9</td>
<td>0.86</td>
</tr>
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</table>

* Student’s t test.

Table 3. Stent types and coronary arteries of stent implantation according to the groups.

<table>
<thead>
<tr>
<th></th>
<th>ISR</th>
<th>CONTROL</th>
<th>TOTAL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DES</td>
<td>13 (32%)</td>
<td>14 (46%)</td>
<td>27</td>
<td>0.23</td>
</tr>
<tr>
<td>BMS</td>
<td>27 (68%)</td>
<td>16 (54%)</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Coronary Artery of Implantation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>13 (33%)</td>
<td>8 (25%)</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>CX</td>
<td>5 (13%)</td>
<td>4 (15%)</td>
<td>9</td>
<td>0.87</td>
</tr>
<tr>
<td>LAD</td>
<td>22 (54%)</td>
<td>18 (60%)</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test.

Table 4. Stent types and coronary arteries of stent implantation according to the groups.

<table>
<thead>
<tr>
<th>Stent Type</th>
<th>ISR</th>
<th>CONTROL</th>
<th>TOTAL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES</td>
<td>13 (32%)</td>
<td>14 (46%)</td>
<td>27</td>
<td>0.23</td>
</tr>
<tr>
<td>BMS</td>
<td>27 (68%)</td>
<td>16 (54%)</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In our study, serum prolidase level was found to be significantly higher in the ISR group compared to the control group. In the correlation analysis, prolidase level was correlated with the rate of restenosis. In the linear regression analysis, we found that serum prolidase level and Gensini’s score were independent predictors of stent restenosis. In-stent restenosis is defined as the gradual re-narrowing of the lesion in the stent in the coronary artery as a result of arterial damage and subsequent neointimal tissue proliferation [15]. It has been shown that coronary artery stent restenosis is a negative response caused by a decrease in vascular diameter due to damage occurring during coronary revascularization [8]. Neointimal hyperplasia is known to be the most important mechanism underlying ISR [16]. Neointimal hyperplasia is associated with collagen synthesis and matrix proteins. Previous studies have shown that, because of the wide tissue distribution, changes in serum prolidase enzyme levels may play a role in the development and outcome of many diseases and is directly associated with the synthesis of collagen, which is abundantly found in the body [14, 17, 18].

Rabus et al. compared 26 patients who underwent valve replacement due to rheumatic etiology, 24 patients who underwent valve replacement due to degenerative etiology and 20 healthy volunteers in terms of prolidase enzyme levels. Serum level of prolidase enzyme was statistically significantly higher in the control group than in the patient group (p<0.001). No significant difference was observed between rheumatic and degenerative valve replacement groups in terms of prolidase enzyme levels. There was no significant correlation between serum prolidase level and severity of valvular disease (p=0.05). The authors stated that rheumatic and degenerative valvular diseases were associated with decreased serum prolidase enzyme level [19].

In a study by Akturk et al. evaluating serum prolidase levels in 40 patients with coronary artery ectasia (CEA) and 40 control subjects with angiographically normal coronary arteries, serum prolidase level was found to be significantly higher in CEA patients and was an independent predictor of CEA [20]. In another study, serum level of prolidase enzyme was compared between the patients with ischemic and idiopathic dilated cardiomyopathy (DCM) and healthy individuals, and prolidase enzyme levels were found to be significantly lower in patients with ischemic DCM compared to the other two groups. The authors stated that the results obtained from this study were the opposite of those predicted. Normally, serum prolidase level is expected to be higher in patients with ischemic etiology, and the authors proposed that lower serum prolidase levels found in patients with ischemic etiology may be caused by decreased collagen cycle in heart tissue and decreased physical activity in these patients [21].

Demirbag et al. compared hypertensive patients with and without left ventricular hypertrophy and healthy individuals. In that study, serum prolidase level was significantly higher in the hypertensive patient groups and was associated with the duration of hypertension [22].

In a study evaluating CAD patients, Yildiz et al. Compared 199 patients with severe CAD and 122 healthy individuals. Serum prolidase activity was found to be significantly higher in the CAD group (52.5±5.6 U/L vs 46.7± 5.1 U/L, p<0.001). On the other hand, the authors stated that serum prolidase level was an independent predictor of CAD [23].

In a study by Suner et al., the authors stated that increased prolidase activity may contribute to the development of slow coronary flow [24]. As we mentioned so far, prolidase enzyme levels have been...
evaluated in various cardiac conditions such as valvular diseases, hypertension and dilated cardiomyopathy, although there are no sufficient studies about the value of prolidase in patient groups with CAD and stent restenosis. Therefore, in the present study, we found significantly higher serum levels of prolidase in the patient group than in the control group (993.18± 609.99 µM/mL vs 468.5± 258.92 µM/mL, p= 0.02).

In the current study, we demonstrated that serum prolidase levels were moderately correlated with the severity of stent restenosis. On the other hand, we found that serum prolidase level was an independent factor in predicting the severity of in-stent restenosis ($\beta$ = 0.45, p = 0.02).

We think that the underlying reason for the high serum prolidase level in patients with stent restenosis was associated with the prolidase's direct role in collagen synthesis and thus, its indirect role in the development of neointimal proliferation.

Study Limitations

Relatively small number of patients, lack of sufficient data about PCI procedures performed in patients (diameters and sizes of stents, duration and pressure of balloon inflation etc.) and the lack of evaluation of other biomarkers that play a role in the collagen mechanism are the main limitations of this study.

Conclusion:

The data obtained in this study indicate that serum prolidase enzyme level is closely related with in-stent restenosis and is an independent factor in predicting the severity of in-stent restenosis.

Our results suggest that serum prolidase enzyme levels will enable early and correct evaluation of stent restenosis in patients undergoing PCI with coronary stents and will contribute to determining the time of altering or intensifying the existing treatment. Further comprehensive clinical studies with a large number of patients are needed to support our results.

Acknowledgment

We would like to thank Mehmet Tosun, MD from Abant Izzet Baysal University Medical Faculty, Department of Biochemistry for his valuable support in biochemical analyses.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: None

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References
