The relationship between coronary collateral artery development and inflammatory markers

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ABSTRACT

Objective: This study aims to show the effect of myeloperoxidase (MPO), hsCRP, TNF-alpha values and leukocyte count on the development of coronary collateral arteries in patients with severely diseased coronary arteries.

Methods: Current study is an observational cross-sectional study. In the study, 295 patients who had functional obstruction or total coronary occlusion at least 1 month on their angiograms were included. We divided the study population into two groups according to their collateral grade as good collateral (Group 1) (169 patients) and poor collateral (Group 2) (126 patients). Multiple logistic regression analysis was used for independent variables associated with the coronary collateral grade.

Results: History of stable angina pectoris was statistically more prevalent in good collateral group (61.5% and 48.4%, p=0.025). Furthermore, MPO activation was higher in good collateral group and the difference was statistically significant (3.7 U/mL and 3.0 U/mL p=0.001). In multiple logistic regression analysis, stable angina pectoris [OR 1.7, 95% CI (1.05-2.8), p=0.03] and high MPO levels [OR 2.7, 95% CI (1.7-4.3), p<0.001] were found to be independent predictors of good collateral development.

Conclusion: We think that proinflammatory enzymes and cytokines released from these cells rather than inflammatory cells themselves may play an important role on the collateral development. (Anadolu Kardiyol Derg 2014; 14: 336-41)

Key words: collateral arteries, inflammatory markers, stable angina pectoris, angiogenesis, regression analysis.

Introduction

Atherosclerotic heart disease is the most frequent cause of death in adults. Recent advances in the modernization of coronary intensive care units, the use of beta blockers, coronary bypass surgery, fibrinolytic therapy and primary coronary angioplasty and similar treatment methods has resulted in important declines in mortality rates due to coronary heart disease (1, 2). A well-developed collateral artery also restricts the infarction area during acute myocardial infarction (MI) and prevents the occurrence of Q wave MI, left ventricular aneurysm and coronary failure. As is known, inflammation has a significant role in the progression of atherosclerosis. For this reason, an examination of inflammatory markers that can affect the development of collateral arteries is important (2-7).

The imbalance between oxygen requirements as a result of coronary artery stenosis or coronary artery occlusion lead to an increase in coronary collateral development in humans, dogs and pigs. The formation of coronary collaterals takes the form of de novo “angiogenesis” with the budding of new capillaries in existing blood vessels or as “arteriogenesis” that is present as a result of the development and maturing of anastomosis vessels in existing arteries since birth (6). In both cases endothelial cells and the growth factors secreted from inflammatory cells such as thrombocyte and monocyte that attack the ischemic region play an important role (6-8).

The tumor necrosis factor α (TNF-alfa) is a proinflammatory cytokine that is produced from leading macrophages. It increases the expression of adhesion molecules (such as VCAM-1 and ICAM-1). And also by increasing the production of nitric oxide (NO) and prostacycline from endothelium, it increases blood flow and creates local vasodilatation (9-14).

CRP is one of the marker of vascular inflammation. Some studies show inverse graded association between CRP and the presence of coronary collaterals in patients with stable angina pectoris (15-18). In our study there is no relation between hsCRP...
and collateral development. White blood cells are one of the important cells that give response to inflammation. Widely used, stable and with better standardization compared to other inflammatory indicators, white blood cell measurement is considered as an independent predictor of smoking, age etc. in cardiovascular mortality (19).

Myeloperoxidase (MPO) is stored inside azurophilic granules within polymorphic nuclei neutrophils and macrophages (20). MPO, through hydrogen peroxide reaction, forms permeable oxidative substances that contains free radicals, antimicrobial activities and causes oxidative damage in tissues (20-23). It is elevated in coronary artery disease and higher levels of MPO is found with progression of coronary artery disease (CAD) (21).

It has been shown that TNF-alfa and through nitric acid oxide synthesis of high sensitive C-reactive protein (hsCRP), cytokines and development factors regulates angiogenesis (12). But there is no sufficient data in the relationship between MPO, white cells and collateral development. There are interindividual differences in the number and extent of collateral vessels among patients with a similiar degree of coronary atherosclerosis (4). This is due to environmental and genetic disparities. In this study, we have aimed in identifying the inflammatory markers such as MPO serum, TNF-alfa, hsCRP and white blood cell count which we consider to effect the collateral development.

Methods

Study design
This study has a cross-sectional and observational design.

Study population
The study population consisted of 295 patients who underwent clinically indicated coronary angiography in our department and were found to have at least one major coronary occlusion, or a stenosis of $\geq 95\%$ with Thrombolysis In Myocardial Infarction (TIMI) grade $\leq 1$ anterograde flow between October 2008 and April 2009. All patients had stable anginal symptoms and/or positive stress test results or electrocardiographic changes indicating ischemia.

Study protocol
Clinical characteristics of the patients including age, weight, gender and any data known to influence development of collaterals such as current medications, history of hypertension, diabetes mellitus, complete blood count and fasting glucose levels were documented. Patients with a history of coronary bypass surgery were excluded if the operation has been done within the past 30 days and the distal aspect of the qualifying severely stenosed or occluded artery is supplied by the patent bypass graft. Patients who had percutaneous coronary intervention within 30 days, acute or chronic infectious disease and malignancy were also excluded. All patients gave written informed consent and local Ethics Committee approved the study protocol.

Baseline clinical examination
Routine blood samples were taken from the patients who were admitted to hospital for coronary angiography after overnight fasting. Patients with low density lipoprotein (LDL) levels greater than 100 mg/dL or under lipid lowering drug therapy were considered to have hyperlipidemia. Patients with systolic or diastolic blood pressure $\geq 140$ mm Hg or 90 mm Hg or who were under antihypertensive treatment were considered to be hypertensive (based on the mean of the two readings). Patients who were being treated for diabetes mellitus (DM) or who had a fasting glucose concentration $\geq 126$ mg/dL were considered to have DM. Smoking habit was recorded as one that has lasted for more than 1 year. The Friedewald’s formula was used for LDL cholesterol measurement.

Laboratory analyses
5 mL of blood samples were collected from the arterial sheaths immediately following coronary angiography from each patient and vacutiner tubes centrifuged at 5000 rpm for 15 minutes within 1 hour. Then plasma collected and stored at -20˚C. MPO was measured with enzyme linked immunosorbent assay (ELISA) (Imtec MPO ANCA. Wiesbaden-Germany) and TNF-alpha was measured with ELISA (Biosource, Carlsbad, California, USA) in immunology laboratuary. hsCRP was measured with nephelometry (CardioPhase hsCRP BN II nefelometre, Dade- Behring-Germany) and white blood cell counts were measured in the Beckman Coulter LH 750 automatic blood count analyzer by the VSC prinsible in Ankara University Ibni-Sina hospital central laboratuary.

Coronary angiography and grading of coronary collaterals
Coronary angiography was completed by two experienced cardiologist blinded to the study (Philips Integris 3000 system Philips, Holland) according to the Judkins technique using automated quantitative coronary artery stenosis assessment program of same system. The diseased vessel was identified as stenosis of the major coronary artery of at least 75%. Collateral flow was graded according to the Rentrop classification (6) and based on the injection that best opacified the occluded vessel: grade 0, no visible filling of any collateral vessels; grade 1, filling of side branches of the artery to be perfused by collateral vessels without visualization of the epicardial segment; grade 2, partial filling of the epicardial segment by collateral vessels; and grade 3, complete filling of the epicardial segment by collateral vessels. According to this classification we divided 295 patients into two groups. Grade 0 and 1 were named as poor collateral (Group 2), grade 2 and 3 were named as good collateral (Group 1). In subjects with $\geq 1$ collateral supplying the distal aspect of the diseased artery, the higher collateral grade was used. In subjects with $\geq 1$ qualifying severely diseased vessel, the vessel with the higher collateral grade was chosen for analysis.

Statistical analysis
Categorical variables were defined as percentage, continuous variables were presented as mean±standard error. Comparisons
among groups were tested for normal distribution with student-t test, for abnormal distribution with Mann-Whitney U test. Relationship between continuous variables that were not normally distributed and collateral grade was tested with Spearman correlation test. History of stable angina pectoris and serum MPO level were found to be significantly correlated with collateral grade. Therefore, these variables were used in a multiple logistic regression analysis as independent variables to determine their effect on collateral grade. Statistical significance was defined as p<0.05. The SPSS statistical software (SPSS for windows 11.5, SPSS Inc, Chicago, IL) was used for all statistical calculations.

**Results**

**Clinical characteristics of the population**

The patient population consisted of 218 males (74%) and 77 females (26%) (mean age, 63±10 years). The prevalence of various demographic, angiographic and therapy related characteristics of the study subjects at the time of coronary angiography according to collateral classification are shown in Table 1. Of the 295 patients, 169 had good collaterals (grade 3 and grade 2), whereas 126 had poor collaterals (grade 1 and grade 0). Subjects with good collaterals were significantly more likely to have stable angina pectoris history (p=0.025) and the prevalence of RCA (right coronary artery) lesion was higher in the good collateral group (p=0.02) whereas CX (circumflex artery) lesion was higher in the poor collateral group (p=0.001). The prevalence of all other cardiovascular risk factors and use of medications were similar at different levels of collaterals.

**Table 1. Characteristics of the subjects according to collateral groups**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=169)</th>
<th>Group 2 (n=126)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years</td>
<td>63±10.6</td>
<td>63±10.2</td>
<td>0.996</td>
</tr>
<tr>
<td>Gender (M/F), n</td>
<td>127/42</td>
<td>91/35</td>
<td>0.571</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>124 (73.4)</td>
<td>91 (72.2)</td>
<td>0.826</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>64 (37.9)</td>
<td>48 (38.1)</td>
<td>0.969</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>62 (36.7)</td>
<td>44 (34.9)</td>
<td>0.755</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>84 (49.7)</td>
<td>59 (46.8)</td>
<td>0.625</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
<td>65 (38.5)</td>
<td>56 (44.4)</td>
<td>0.301</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>76 (45)</td>
<td>57 (45.2)</td>
<td>0.964</td>
</tr>
<tr>
<td>History of stable angina pectoris, n (%)</td>
<td>104 (61.5)</td>
<td>61 (48.4)</td>
<td>0.025</td>
</tr>
<tr>
<td>Occluded coronary artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD, n (%)</td>
<td>38 (22.5)</td>
<td>36 (28.6)</td>
<td>0.233</td>
</tr>
<tr>
<td>CX, n (%)</td>
<td>15 (8.9)</td>
<td>30 (23.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RCA, n (%)</td>
<td>120 (71.0)</td>
<td>60 (47.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Beta-blockers, n (%)</td>
<td>120 (71)</td>
<td>91 (72.2)</td>
<td>0.819</td>
</tr>
<tr>
<td>ACE-I or ARBs, n (%)</td>
<td>138 (81.7)</td>
<td>93 (73.8)</td>
<td>0.106</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>91 (53.8)</td>
<td>70 (55.6)</td>
<td>0.771</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>164 (97.0)</td>
<td>125 (99.2)</td>
<td>0.193</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>126 (74.6)</td>
<td>103 (81.7)</td>
<td>0.143</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>107.5±39.7</td>
<td>107±38.6</td>
<td>0.912</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>178±45.5</td>
<td>181.5±45.8</td>
<td>0.515</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>41.1±26.7</td>
<td>40.2±12.2</td>
<td>0.719</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>153.6±80.4</td>
<td>171.2±115.6</td>
<td>0.328</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>120.6±60.5</td>
<td>124.5±66.9</td>
<td>0.979</td>
</tr>
</tbody>
</table>

Data are presented as number (percentage) and mean±SD values

Group 1: good collateral, Group 2: poor collateral

* Student-t test and Mann-Whitney U test

**Table 3. Predictors of good collateral arteries**

<table>
<thead>
<tr>
<th></th>
<th>*P value OR CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of stable angina pectoris</td>
<td>0.03 1.7 1.05-2.8</td>
</tr>
<tr>
<td>MPO, &gt;2.91 U/mL</td>
<td>&lt;0.001 2.7 1.7-4.3</td>
</tr>
</tbody>
</table>

*Multiple logistic regression analysis

Dependent variable collateral grade, independent variables-history of stable angina pectoris, serum MPO level

MPO - myeloperoxidase

**Table 2. Relationship between inflammatory markers and collateral**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=169)</th>
<th>Group 2 (n=126)</th>
<th>*P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP, mg/L</td>
<td>37.9±17.2</td>
<td>25.8±47.4</td>
<td>0.940</td>
</tr>
<tr>
<td>MPO, U/mL</td>
<td>120 (71)</td>
<td>91 (72.2)</td>
<td></td>
</tr>
<tr>
<td>ACE-I, MPO and hsCRP levels, TNF-alpha, MPO and hsCRP levels</td>
<td>0.106</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The relationship of collateral grade and TNF-alpha, MPO and hsCRP

TNF-alpha, MPO, hsCRP levels, white blood cell counts and subgroup levels were shown in Table 2. hsCRP, TNF-alpha levels, white blood cell counts and subgroup levels were not significantly different among groups (p=0.05) (Table 2). Furthermore MPO levels were significantly higher in the good collateral group than poor collateral group (3.7 U/mL vs. 3.0 U/mL, p=0.001) (Table 2, Fig. 1). We also found very weak correlation between the MPO levels and collateral grade albeit p value was significant (p=0.009, r=0.15) (Fig. 2). In multiple logistic regression analysis, stable angina pectoris [odds ratio 1.7, 95% confidence interval (1.05-2.8), p=0.03] and high MPO levels [odds ratio 2.7, 95% confidence interval (1.7-4.3), p<0.001] were found to be independent predictors of good collateral development (Table 3).
Discussion

In this study, we aimed to identify possible determinants of coronary collaterals in patients with severe coronary artery disease. We found that stable angina pectoris and MPO levels were independent predictors of development of coronary collaterals.

The presence of collateral vessels protects the heart from myocardial ischemia and infarction, and following an infarction by enabling the development of less Q wave MI, left ventricular aneurysm and coronary failure. So they provide a positive effect in in-hospital and long-term prognosis (1). An increased shear stress following serious arterial blockage arteriogenesis is stimulated. In this mechanism it is thought that MCP-1 (monocyte chemotactic protein-1) is effective. While shear stress is at a maximum at the beginning, this stress gradually decreases as the diameter of collateral arteries expands (5, 7, 8). Assuming that hypertension plays a facilitating role in this mechanism, we can consider it to have a positive effect on collateral development. In our study, hypertension in the good collateral group was also detected more but this difference was not found to be statistically significant.

We know that coronary collateral growth is impaired in type 2 diabetes mellitus and metabolic syndrome (24). In our study, 112 diabetic patients were present and in terms of DM frequency, no significant difference was determined among the two groups. This may due to insufficient number of patients and undetectable arteries formed by angiogenesis with coronary angiography. Additionally, a comparison of the number of diseased arteries between the two groups was not carried out in this study.

Although many studies have suggested that statins and nitrates may promote vessel growth, clinical study results are generally lacking (8, 25). We did not find any relation between coronary collateral and use of this group of drugs.

In the latest studies conducted, it has been suggest that CRP results in differentiating endothelial precursor cells, that it badly effects survival and that it disrupts the angiogenic and arteriogenic functions of the cells (15, 18). Whereas in the study carried out by Turu et al. (16) CRP stimulated in angiogenesis. Even though there are studies that show an inverse relationship between collateral artery development and high CRP levels (15), we did not determine a meaningful relationship between collateral development and hsCRP in our study.

Barron et al. (26) in a study conducted on 975 patients found a correlation between high blood cell counts, increased thrombus load, increased coronary failure and incidences of death after acute myocardial infarction. Even though there is no study available that indicates the relationship between coronary collateral development and white cell counts, we compared white cell counts between good and bad collateral arteries and found no significant difference.

TNF-alfa is an important cytokine that plays a role in inflammation. Its effects on the cardiovascular system are currently being researched. Numerous studies have shown that TNF-alfa reduces eNOS production in various arteries thus leading to a decline in NO production (12-14). In a study carried out by Luo et al. (10) TNF-alfa through p55 receptors affected arteriogenesis in a positive way. In our study we considered TNF-alfa having an effect on angiogenesis. So we compared TNF-alfa levels with patients that have good and bad collateral but could not determine a significant difference.

Carrão et al. (27) found that granulocyte-colony stimulating factor (G-CSF) directly stimulates cardiomyocytes and promote angiogenesis. Besides this, there are many studies that indicate the important role played by monocytes and macrophages in collateral development where MPO is stored (20, 21, 28). Kocaman et al. (29) divided 210 patients, where coronary artery
stenosis of these patients was 95% or above and did not have diabetes, into two groups of good and bad collateral and found that the group with good collateral had a high monocyte count and that the monocyte count was an independent predictor in the development of good collateral. In our study it was determined that while in terms of white cell count, a noticeable difference was not found among the two groups, the MPO level was found to be high among patients with good collateral and that this difference was statistically meaningful. We interpreted the high level of MPO in particular as due to the possibility of an increase in MPO activity in neutrophiles and monocytes. And G-CSF may also increase MPO activity but in our study we did not measure the serum G-CSF level. Additionally, after a multiple logistic regression analysis is carried out, a high MPO level was determined to be the independent predictor of good collateral artery development. Even though there are no head to head studies that show the relationship between collateral artery development and MPO levels in the literature, we consider that in collateral development rather than inflammatory cells, the proinflammatory enzymes and cytokines secreted from these cells can play a more important role.

Study limitations

We consider that low number of subject and unknown duration of the medication were the two limitations of this study. That’s why having diabetes mellitus and use of medication were not significantly different among groups. Additionally we performed all measurements at one point in time. HsCRP, MPO, blood cell count and TNF-alpha are inflammatory markers and we tried to find out the predictive value of them measured at one point in time to detect the relation of the presence of collateral circulation. Design of our study reflected routine daily practice in the majority of cardiology clinics. Repeated angiography assessments may be needed but due to the invasive nature of coronary angiography we did not perform.

Conclusion

The findings from our study leave us to think that while TNF-alfa, hsCRP and white cell count does not have a meaningful effect on collateral development, MPO levels has a positive effect on collateral development. But randomized clinical studies with follow up are required to evaluate the role of them on cardiovascular outcomes.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.


References