EVALUATION OF THE LEVELS OF METALLOPROTEINSASE-2 IN PATIENTS WITH ABDOMINAL ANEURYSM AND ABDOMINAL HERNIAS*

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Abdominal aortic aneurysms and abdominal hernias become an important health problems of our times. Abdominal aortic aneurysm and its rupture is one of the most dangerous fact in vascular surgery. There are some theories pointing to a multifactoral genesis of these kinds of diseases, all of them assume the attenuation of abdominal fascia and abdominal aortic wall. The density and continuity of these structures depend on collagen and elastic fibers structure. Reducing the strength of the fibers—may be—due to changes in the extracellular matrix (ECM) by the proteolytic enzymes—matrix metalloproteinases (MMPs) that degrade extracellular matrix proteins. These enzymes play an important role in the development of many disease: malignant tumors (colon, breast, lung, pancreas), cardiovascular disease (myocardial infarction, ischemia-reperfusion injury), connective tissue diseases (Ehler-Danlos Syndrome, Marfan’s Syndrome), complications of diabetes (retinopathy, nephropathy). One of the most important is matrix metalloproteinase-2 (MMP-2).

The aim of the study was an estimation of the MMP-2 blood levels in patients with abdominal aortic aneurysm and primary abdominal hernia, and in patients with only abdominal aortic aneurysm.

Material and methods. The study involved 88 patients aged 42 to 89 years, including 75 men and 13 women. Patients were divided into two groups: patients with abdominal aortic aneurysm and primary abdominal hernia (45 persons, representing 51.1% of all group) and patients with only abdominal aortic aneurysm (43 persons, representing 48,9% of all group).

Results. It was a statistically significant increase in MMP-2 blood levels in patients with abdominal aortic aneurysm and primary abdominal hernia compared to patients with only abdominal aortic aneurysm. It was a statistically significant increase in the prevalence of POCHP in patients with only abdominal aortic aneurysm compared to patients with abdominal aortic aneurysm and primary abdominal hernia.

Conclusions. Statistically significant higher MMP-2 blood levels in patients with abdominal aortic aneurysm and primary abdominal hernia seems shows that this enzyme plays a role in the pathogenesis of primary abdominal hernias. The observed distribution of MMP-2 blood levels in patients with abdominal aortic aneurysm and primary abdominal hernia may raise the conclusion that this enzyme determines the presence of multi-organ failure of the connective tissue – the patients with only abdominal aortic aneurysm had significantly lower MMP-2 blood levels.

Key words: abdominal aortic aneurysm, abdominal hernia, matrix metalloproteinases (MMP-s), extracellular matrix (ECM), fascia

According to the Great Medical Dictionary, aneurysm is a pulsating tumour, formed as a result of a widening of the aorta along its section, usually as a consequence of some pathological changes, with a shape of a spindle or eccentrically positioned sac filled with blood or

* A summary of a doctoral thesis
thrombus (1). In an aneurysm, the lumen of the aorta is widened by at least 50% compared to the unchanged section above it (2). The process of formation of an aneurysm is associated with several intertwining and complementary pathophysiological mechanisms (3–6). The pathological changes in the aortic wall, which result in its weakening, are caused by disorders of elastin and collagen metabolism. These are the most important structural proteins in an elastic-type artery, of which the aorta is an example. Elastin is not produced in the aorta of an adult and, being a stable protein, it undergoes hardly any metabolic changes in an adult. Half-life of the protein is about 70 years (2-6).

Collagen, the other structural protein of the aortic wall, mainly type I and III, is synthesized by cells of the smooth muscles of the aorta throughout a human life (2). The wall of a healthy aorta produces more type I than type III collagen – the amount of the former is twice or three times as large as that of the latter – whereas the ratio is disturbed in the wall with an aneurysm (6). Collagen fibres are highly resilient and – unlike elastic ones – they do not stretch. It is they that make the walls of a vessel mechanically resistant. Increased decomposition of collagen fibres, out-of-proportion to their stored amount in the endarterium and intima tunica, results in an increase and, consequently, a rupture of the aneurysm (7). Collagen fibres are over 20 times tougher than elastin and 300 times less resilient (2-7). The structural properties of collagen and elastin are complementary. Formation, growth and rupture of an aneurysm is indicative of a disorder of the function of those two proteins. Collagen and elastic fibres are the base of the structure not only of the arterial walls. They are components of all the fascial elements of the abdominal wall, which include oblique, outer, inner and transverse abdominal aponeuroses, superficial and transverse abdominal fascia.

From the histological point of view, fascia and aponeuroses consist of connective tissue, which performs three main functions: it is the stroma and physical protection of some organs and tissues, it transports nutrients and metabolites and it is a protective barrier of the body. Those functions determine its general structure. It comprises cells and plenty of extracellular matrix, which consists of ground matrix and fibres (mainly collagen, reticular and elastic fibres). The continuity and cohesion of fascia is structurally dependent on the properties of collagen fibres and on mutual proportions of collagen I and III, with collagen I dominating. The main biological mechanism which results in weakening of the structure of connective tissue and, consequently, development of hernia, is a pathology of connective tissue or its mechanical weakening by a post-operative scar.

In both cases, but to a different extent, disturbed enzymatic balance of extracellular matrix start to play a role – more specifically: disturbance of metabolism of collagen, which accounts for over 80% of the dry matter of the connective tissue of abdominal fascia. The mechanism is the most apparent in primary abdominal hernias.

Symptomatic mutations of genes encoding collagen proteins and structural proteins of elastic fibres give rise to a variety of forms of genetically conditioned pathological syndromes, whose components are aneurysms and hernias. The diseases include Ehler-Danlos Syndrome and Marfan’s Syndrome. Its features include a systemic, but strongly expressed defect of connective tissue, which results in specific irregularities in other systems, such as the bone and joint system and the visual system. However, in majority of patients with aneurysms or primary abdominal hernias there are no visible defects in connective tissue, and weakening of the aortic wall and abdominal fascia is a symptom of degeneration of connective tissue (8).

Pathology of the metabolic processes in the extracellular matrix of connective tissue is manifest mainly as increased proteolytic activity of enzymes – matrix metalloproteinases, which leads to disturbing a specific equilibrium between decomposition and production, mainly of collagen and elastic fibres, which exists in healthy tissues. These enzymes play an important role in the development of many diseases: malignant tumors (colon, breast, lung, pancreas), cardiovascular disease (myocardial infarction, ischemia-reperfusion injury), connective tissue diseases (Ehler-Danlos Syndrome, Marfan’s Syndrome), complications of diabetes (retinopathy, nephropathy). One of the most important enzymes in the group is metalloproteinase-2, because of a diversity of substrates which it transforms (those found so far include: collagen type I, IV, V, VII, X, XI, XIV, gelatin, fibronectin, laminin, aggrecan, casein), which
can make many pathological processes attributable to the extent of its activity.

Metalloprotease-2 (9) plays an important role in degeneration of the matrix, especially its fibres. The range of its activities is not limited to the skin or abdominal fascia. It also plays a role in degenerative processes of the abdominal aortic wall, which results in formation of an aneurysm. These observation allow a conclusion that enzymatic disorders responsible for a defect of connective tissue are of a systemic nature, which is not always reflected in groups of symptoms with the genetic etiology, such as Ehlers-Danlos syndrome, Marfan’s syndrome, osteogenesis imperfecta, cutis laxa (10), but also in patients with abdominal aneurysm, colonic diverticular disease, stress incontinence, inguinal hernia.

The aim of the study was to estimate the MMP-2 blood levels in patients with abdominal aortic aneurysm and primary abdominal hernia, and in patients with abdominal aortic aneurysm alone.

**MATERIAL AND METHODS**

Two groups of patients were included in the study. In the first group, a retrospective analysis was performed of the patients operated on at the Department of General and Vascular Surgery in 2004-2007 for abdominal aortic aneurysm. Patients with abdominal aortic aneurysm with the diameter of 5 cm or larger were eligible for the operation. The other, prospective group, included patients with abdominal aneurysm, being in the care of the Clinic of Peripheral Vascular Diseases and patients admitted to the Department for an abdominal aortic aneurysm surgery according to the same criteria of operative treatment.

The patients were divided into two subgroups: those with primary abdominal hernia (some of the patients after a hernia surgery and some with unoperated hernia) – 45 people, and patients without abdominal hernias – 43 people. There were 88 people in the study population aged 42 to 89 years, including 75 male and 13 female patients. The study subjects were asked to complete a questionnaire, with such information required as age, sex, beginning of the disease, concomitant diseases (ischaemic heart disease, chronic obstructive pulmonary disease, arterial hypertension, brain stroke, transient paroxysmal loss of consciousness, intermittent dysbasia, diabetes, connective tissue diseases, abdominal hernias, iliac aortic aneurysm), nicotinism, aneurysms, hernias, connective tissue diseases in family members, previous surgeries (especially abdominal hernia surgeries). Blood levels of metalloproteinase-2 were determined.

Venous blood for analysis was collected 24 hours before the planned surgery from the patients admitted to the Department in order to perform a surgery of abdominal aortic aneurysm and during a visit to the Clinic from the patients being in its care because of abdominal aortic aneurysm. The blood was centrifuged and the serum was frozen in containers made of polypropylene at the temperature of –24 degrees Celsius. The level of metalloproteinase-2 was determined by the ELISA method, with RD Systems kits, at the Department of Immunoendocrinology of the Medical University in Łódź.

Descriptive methods and statistical reasoning methods were used to analyse the data collected during the study.

The stratum weight was calculated in descriptions of each patient, including in subgroups: with abdominal aortic aneurysm and abdominal hernia and in patients with abdominal aortic aneurysm alone. The stratum weight was expressed as percentage in the group considered as a whole and it was left as fractions in the analysed subgroups, which was caused by the size of the subgroups which included 45 and 43 units. The following characteristics were calculated for the measurable attributes: arithmetic mean ( ), median (Me), standard deviation (SD) and variability coefficient; the minimum and maximum value was also given. The arithmetic mean and the median express the average level of an effect, while the standard deviation and the variability coefficient express the dispersion of the results.

The chi² independence test was performed when comparing the frequency of different varieties of attributes in subgroups. If, when the value of the chi² test were calculated, some cells of the table contained values smaller than 5, than the Yates’ correction was applied when the test value was calculated.

Before the mean values of the age and of the MMP-2 concentration were compared, it was verified whether the distributions of the
attributes do not significantly deviate from the normal distribution. To this end, the λ-Kolmogorov test was performed. Since the distribution of neither of the measurable attributes analysed in the study deviated significantly from the normal distribution, the parametric test was used in comparisons. A test for comparing mean values of large samples was performed (the number of subjects in both groups were > 30 units), based on the normal distribution.

Those differences between frequencies (or mean values) were regarded as significant for which the value of the chi-square (or z) test proved equal to or higher than the critical value as read from the relevant tables, for the appropriate number of degrees of freedom, at the error probability p<0.05.

RESULTS

The blood level of MMP-2 in the whole group of patients ranged from 158 to 627 ng/mL, with the mean value of equal to 370.7 ± 104.3 ng/mL; it exceeded 365 ng/mL in half of the patients. When all the patients were divided into two groups: those with abdominal aortic aneurysm and with abdominal hernia and those with abdominal aortic aneurysm alone, it turned out that there was a statistically significant difference in the level of MMP-2 between them (p<0.01). A significantly higher level was observed in the group with abdominal aortic aneurysm and abdominal hernia than in the group with aneurysm alone; the mean values in those groups were, respectively: 400.7 ± 104.3 ng/mL and 339.3 ± 96.9 ng/mL. In the first group, the level exceeded 382 ng/mL in half of the patients, while in the other it was lower than 350 ng/mL in half of the patients (tab. 1 and 2, fig. 1).

Most of the analysed concomitant diseases occurred in the comparable groups of patients with similar frequency. This was not the case for the COPD, which was observed more frequently in patients with abdominal aortic aneurysm alone (without abdominal hernia), p<0.05. A considerable difference, though not a statistically significant one, was observed in the incidence of brain stroke, which occurred more frequently in patients with abdominal aortic aneurysm and abdominal hernia than in the group with aneurysm alone (tab. 3).

DISCUSSION

One of the major factors in the etiopathogenesis of abdominal aortic aneurysm and primary abdominal hernia is a defect of connective tissue which involves increased degradation of collagen and elastic fibres (7, 11, 12). Collagen is a non-uniform protein. Currently, 29 types of the protein have been identified, distributed in a diverse manner in different tissues of the body and produced in different quantities. One of the features of collagen fibres is their great strength – 10 kg/1 mm of a fibre diameter. The most important factor in terms of the structure and mechanical strength of abdominal fascia and, consequently, the pathogenesis of abdominal hernias, is the collagen I/collagen III ratio (11, 12). Collagen type I is regarded as the mature form, with thick

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calculated parameters of MMP-2 (ng/mL)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>max.</td>
</tr>
<tr>
<td>Whole group</td>
<td>158</td>
<td>627</td>
</tr>
<tr>
<td>Abdominal aortic aneurysm and abdominal hernia</td>
<td>203</td>
<td>604</td>
</tr>
<tr>
<td>Abdominal aortic aneurysm</td>
<td>158</td>
<td>627</td>
</tr>
</tbody>
</table>

Comparison of the mean values: z=2.872; p<0.01

Fig. 1. Mean level of MMP-2 in comparable groups

<table>
<thead>
<tr>
<th>Abdominal aortic aneurysm and abdominal hernia</th>
<th>Abdominal aortic aneurysm</th>
<th>Whole group</th>
</tr>
</thead>
<tbody>
<tr>
<td>400.7</td>
<td>339.3</td>
<td>370.7</td>
</tr>
</tbody>
</table>

Table 1. MMP-2 in patients in the groups under study – mean values and measures of dispersion
and strong fibres, while collagen type III is immature, less strong, present mainly in wounds, in early stages of healing. Collagen type I accounts for 85% of all the collagen in the body, and together with collagen type III – for 95%. The quantitative ratio of the collagen types is important for tissues strength. Collagen fibres are different from elastic ones in that they are not so susceptible to elongation and, therefore, they make tissues strongly resistant to deformations. On the other hand, elastic fibres have some reserve of stretchability and, as a consequence, they guarantee that blood vessel retain the original shape while being highly susceptible to being stretched by blood flow. It is not possible to determine the limit of the role of different types of fibres in different tissues. Fascia and aponeuroses must have a reserve of stretchability, but not so large as vascular walls, especially aortic ones, in which the ratio of elastic to collagen fibres is quite large, with the reverse tendency increasingly predominant with the growing distance from the heart. Abdominal integuments play mainly a static role, keeping the peritoneal cavity with its organ in their place; hence the importance of the network of collagen fibres with their limited stretchability, compared to elastic ones.

Assuming that there are mechanisms of degradation of properly synthesised collagen, studies were taken up in the 1980’s of a group of hypothetical factors which could play a role in the etiology of hernias – matrix metalloproteinases. The enzymes drew the researchers’ attention because they are active in extracellular matrix of connective tissue, therefore they can contribute to development of hernias and aortic aneurysms by degradation of its elements, including collagen and elastic fibres.

Table 2. Structure of the study subjects by MMP-2

<table>
<thead>
<tr>
<th>MMP-2 (ng/ml)</th>
<th>Subjects</th>
<th>abdominal aortic aneurysm and abdominal hernia</th>
<th>abdominal aortic aneurysm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>fraction</td>
<td>n</td>
<td>fraction</td>
</tr>
<tr>
<td>Fewer than 200</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0,07</td>
</tr>
<tr>
<td>201-250</td>
<td>3</td>
<td>0,07</td>
<td>5</td>
<td>0,12</td>
</tr>
<tr>
<td>251-300</td>
<td>4</td>
<td>0,09</td>
<td>7</td>
<td>0,16</td>
</tr>
<tr>
<td>301-350</td>
<td>10</td>
<td>0,22</td>
<td>7</td>
<td>0,16</td>
</tr>
<tr>
<td>351-400</td>
<td>7</td>
<td>0,16</td>
<td>11</td>
<td>0,26</td>
</tr>
<tr>
<td>401-450</td>
<td>5</td>
<td>0,11</td>
<td>7</td>
<td>0,16</td>
</tr>
<tr>
<td>451-500</td>
<td>5</td>
<td>0,11</td>
<td>1</td>
<td>0,02</td>
</tr>
<tr>
<td>501 and more</td>
<td>11</td>
<td>0,24</td>
<td>2</td>
<td>0,05</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>1,00</td>
<td>43</td>
<td>1,00</td>
</tr>
</tbody>
</table>

\( \chi^2=13.699; p<0.05 \)

Table 3. Comparison of the incidence of concomitant diseases in the group of patients with abdominal aortic aneurysm and in the group with aneurysm alone

<table>
<thead>
<tr>
<th>Concomitant diseases</th>
<th>Group</th>
<th>abdominal aortic aneurysm and abdominal hernia</th>
<th>abdominal aortic aneurysm</th>
<th>Value of chi(^2) test</th>
<th>Significance of p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>fraction</td>
<td>n</td>
<td>fraction</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>6</td>
<td>0,13</td>
<td>5</td>
<td>0,12</td>
<td>0,040</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>27</td>
<td>0,60</td>
<td>25</td>
<td>0,58</td>
<td>0,002</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>24</td>
<td>0,53</td>
<td>22</td>
<td>0,51</td>
<td>0,042</td>
</tr>
<tr>
<td>Brain stroke</td>
<td>7</td>
<td>0,16</td>
<td>3</td>
<td>0,07</td>
<td>1,607</td>
</tr>
<tr>
<td>Arterial atherosclerosis</td>
<td>8</td>
<td>0,18</td>
<td>9</td>
<td>0,21</td>
<td>0,140</td>
</tr>
<tr>
<td>COPD</td>
<td>5</td>
<td>0,11</td>
<td>12</td>
<td>0,28</td>
<td>3,980</td>
</tr>
<tr>
<td>Neoplastic disease</td>
<td>6</td>
<td>0,13</td>
<td>5</td>
<td>0,12</td>
<td>0,040</td>
</tr>
<tr>
<td>Iliac arterial aneurysm</td>
<td>1</td>
<td>0,02</td>
<td>2</td>
<td>0,05</td>
<td>0,002</td>
</tr>
</tbody>
</table>
Indeed, except for a decrease in the collage I/collagen III ratio in skin fibroblasts in the patients with inguinal hernia, the level of MMP-1 and MMP-13 was found to increase (11, 12).

Those metalloproteinases were initially regarded as responsible for degradation of type I, II and III collagen and for disturbed collagen I/collagen III ratio in patients with hernia. Subsequent studies did not show any differences in expression of mRNA for these metalloproteinases or differences in expression of the enzymes themselves in skin fibroblasts in patients with hernia compared to the control group. On the other hand, studies of expression of MMP-2 in fibroblast cultures from sections of transverse fascia collected from patients with inguinal hernias revealed remarkable activity of the enzyme, leading to the conclusion that – unlike MMP-1 and MMP-13 – MMP-2 actively participates in degradation of elements of extracellular matrix of the connective tissue of fascia (13, 14).

The observations have been confirmed by assays of MMP-2 blood levels in patients with simple inguinal hernias, showing their remarkable increase. It should be stressed that the assays measured the levels of the active form of MMP-2. Contemporary studies have revealed a correlation between the activity of MMP-2 in tissues and its blood level in patients with hernias, where it is significantly – compared with the control group – higher regardless of the patients’ age, with much higher enzyme levels observed in younger patients with simple inguinal hernias (11, 12, 15, 16, 17). The blood level of MMP-2 and TIMP-2 in patients with inguinal hernias was also tested, with the results showing – as in the studies mentioned earlier – a significantly higher blood level of MMP-2 in those patients compared with the control group and a significantly higher blood level of MMP-2 and TIMP-2 in the patients with recurrent inguinal hernia, leading to the conclusion that metabolic disorders of extracellular matrix is the underlying cause of recurrent hernias (17). This seems to be confirmed by identical disturbance of the collagen I/collagen III ratio in patients with primary hernia and in post-operative scars in patients with recurrent hernia. In the same manner as in healthy tissue, where collagen type I is the primary component, its presence is also remarkable in mature scars, making them resistant to mechanical forces. When the healing process runs properly, collagen type III is present in large amounts only in its initial stages.

The process of formation and growth of an aneurysm is linked to several intertwining and complementary pathophysiological mechanisms. These are: ageing of the vascular walls, chronic inflammatory response, neovascularisation of the aneurysm wall with concurrent release of a cascade of proangiogenic and inflammatory factors, excessive apoptosis of the population of smooth muscle cells, biomechanical factors, oxidative stress and proteolysis of structural elements – elastin and collagen – by matrix metalloproteinase (18). Angiogenesis in the aneurysm wall is stimulated by hypoxia or inflammatory infiltration. The inflammatory cells release proangiogenic factors and growth factors, which in turn induce intensive expression of matrix metalloproteinases, mainly MMP-2 and MMP-9 (18, 19). By degrading elements of extracellular matrix, the enzymes act in three directions: they facilitate migration of endothelial cells to the site of formation of new blood vessels and migration of cells of the inflammatory infiltration and they weaken the aortic wall by depriving it of elastin and collagen. A similar effect on releasing matrix metalloproteinases is exerted by free oxygen radicals, formed in the oxidative stress (20, 21).

The aneuritic abdominal aortic wall was found to contain high levels of both MMP-2 and MMP-9 (22). However, an increased level of MMP-9 was also found in the aortic wall with advanced atherosclerotic process, which resulted in its stenosis or obturation. On the other hand, high levels of MMP-2 are typical only of the aneurysm wall (22). These observations apply to the test of the levels of mRNA and proenzymes of MMP-2 and MMP-9 (22).

The distribution of the MMP-2 blood levels in the patients with aortic aneurysm and abdominal hernia and those with aortic aneurysm alone may reflect the concentration and activity of the enzyme in tissues. This is a hypothesis which in this study has not been corroborated by immunohistochemical tests. However, literature reports have confirmed that an increase in blood level of MMP-2 is correlated with its increased concentration and activity in tissues, which has clinical implica-
tions in the form of aortic aneurysm and primary abdominal hernia.

CONCLUSIONS

The statistical analysis has revealed:

1. Statistically significant increase in blood levels of MMP-2 in patients with abdominal aortic aneurysm and primary abdominal hernia (45 patients, accounting for 51.1% of the study population) compared to the patients with abdominal aortic aneurysm (43 patients, accounting for 48.9% of the study population).

2. No statistically significant differences in the incidence of concomitant diseases: diabetes, arterial hypertension, ischaemic heart disease, brain stroke, atherosclerosis of peripheral vessels, malignant tumour, iliac arterial aneurysm, between the group of patients with abdominal aortic aneurysm and primary abdominal hernia and the group of patients with abdominal aortic aneurysm alone.

3. Statistically significant increase in the incidence of COPD in the group of patients with abdominal aortic aneurysm alone compared to the patients with abdominal aortic aneurysm and primary abdominal hernia.

These conclusions and the analysis of the literature reports lead one to the conclusion that one of the major factors in the etiopathogenesis of abdominal aortic aneurysm and primary abdominal hernia is a defect of connective tissue which involves increased degradation of collagen and elastic fibres by matrix metalloproteinases. Furthermore, significantly higher blood levels of MMP-2 in patients with abdominal aortic aneurysm and primary abdominal hernia may indicate that the enzyme plays an important role in etiopathogenesis of primary abdominal hernias. The distribution of blood levels of MMP-2 in patients with abdominal aortic aneurysm and primary abdominal hernia may indicate that the enzyme plays a role in multiorgan defects of connective tissue. The blood levels of MMP-2 in the patients with aneurysm alone was significantly lower.

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