ASSESSMENT OF ACTIVITY OF MATRIX METALLOPROTEASES 2 AND 9 IN THEIR INTERCONNECTION WITH THE LEVEL OF MAGNESIUM IONS IN THE BLOOD OF PATIENTS WITH CONGENITAL PROLAPSED OF MITRAL VALVE

The paper carries out comparative analysis of metalloproteases (MMP) 2 and 9, TIMP expression indexes and their interconnection with Mg$^{2+}$ ions, glycosaminoglycans (GAG) and glucuronidase activity in the blood of patients with congenital PMV. 86 persons, of them 36 (41.9%) males and 50 (58.1%) females aged 15-25 (19.9±1.42) years, with etiological signs of primary (idiopathic, congenital) prolapsed of mitral valve (PMV), have been studied. The patients with PMV and regurgitation of I-II degree showed decreased level of Mg$^{2+}$ ions that was simultaneously associated with disturbance of collagen forming processes, increased synthesis of atypical low-molecular GAG, that passed into the blood stream.

Keywords: Prolapse of mitral valve, metalloproteases, inhibitors of metalloproteases magnesium, Mg$^{2+}$ ions

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Introduction

Congenital (idiopathic, abnormal) PMV is a genetically determined form of the disease related to the II type of connective tissue dysplasia of the heart valve apparatus according to the classification suggested by New-York Heart Association (NYHA) in 1997 (Kadurina and Gorbunova, 2008). PMV is also considered as insignificant heart abnormality as benign abnormalities and their clinical and prognostic importance is not clear till the present time.

The problems of pathogenesis and progress of the disease are not quite clear. PMV is characterized by various clinical manifestations from benign subclinical forms to polyorgan and polysystemic pathology with progressing course (Martinov and Akatova, 2011; Klemenov, 2005). Most of adolescent patients do not suspect anything about PMV and changes are only revealed in planned examinations at the age of 15-25 years (Soboleva and Suhiih, 2007). PMV is often revealed in children of preschool age and in schoolchildren in physical exertion (Storozhakov, Vereshachin and Malynova, 2001). PMV frequency ranges from 1.8% to 38% (Martinov and Akatova, 2011; Storozhakov, Vereshachin and Malynova, 2001).

Among courses of PMV formation, genetically heterogenic abnormalities of the connective tissue formation and in differentiated (asymptomatic) forms with multifactor mechanisms of the development are considered. Their manifestations are not as clear as in syndromic forms and often remain without adequate attention. Important features of disturbance of the cardiac valve apparatus are structural reconstructions of the connective tissue elements, of extracellular matrix (ECM) of collagen and elastin fibers of glucosamine glucans and proteoglycans amorphous substance (Soboleva and Suhiih, 2007). In recent ten years particular attention of investigators is paid to significance of regulation of ECM fibrillar proteins by magnesium ions (Mg$^{2+}$) and MMP (Frunchart, 2003; Nikitina, Demidova and Radzinskii, 2007). Magnesium Mg$^{2+}$ reduction causes’ injury of endothelium, impairment of spatial organization of collagen and elastin responsible for
formation of ECM components and also enzymes taking part in the process of ibrillogenesis (Zemtsovskiy, 2006-2008). Low Mg\(^{2+}\) concentrations influence on cardiovascular system activity, dysplastic disturbances of the valvular apparatus of the heart, increase of mitral valve prolapsed depth, the degree of mitral regurgitation, the size of the left atrium and frequency of myxomatosis regeneration of the mitral valve prolapsing cusp, heart arrhythmia (Martinov and Akatova, 2011; Forster and Shvarts, 2003). Together with this the disturbance of MMP activity leads to ECM disturbance as a constituent element of stroma that performs supporting function for cells as well as plays dynamic role in metabolic process, effecting cellular proliferation, differentiation, migration apoptosis and angiogenesis and also deposits biologically active factors of growth (Soboleva and Suhii, 2007).

At present it is established that MMP are key factors effecting tissue remodeling. Due to some reasons these proteins are expressed in all tissues in all stages of ontogenesis; they are secreted in intercellular space and function in physiological conditions; their expression is only regulated and activated in the condition of intensive tissue rebuilding. As polyfunctional proteins, MMP take part in the mechanism of apoptosis and angiogenesis. In addition it is known that they are single proteolytic enzymes that can denature fibrillary collagens. Natural MMP opposites are tissue inhibitors metalloproteinase (TIMP) and ECM proteins subfamily, regulating and modeling MMP activity. At present more than 20 MMP and TIMP are defined.

The balance between MMP and TIMP is not constant both in physiological processes, accompanied by growth and development of tissues, and in various pathologies, including in differentiated dysplasia of the connective tissue (Kadurina and Gorbunova, 2008). MMP-2 and MMP-9 are the most widely spread representatives of MMP family. These enzymes are able to split denatured collagens, fibronectin, lamin, entactin, elastin, etc. It was shown that MMP-2 and MMP-9 stimulate invasive processes which are associated with angiogenesis (IP) due to their ability to hydrolyze fibrillary collagens of the 4\(^{\text{th}}\) type. Comparatively small molecular mass and solubility in biological fluids provide ability for MMP and TIMP to pass into the blood serum in the amount, proportional to the tissue expression. That is why change of MMP and TIMP levels is considered as possible prospective biological markers of differential diagnostics, prognosis and treatment of connective tissue dysplasia (Zemtsovskiy, 2008).

In connection with the above mentioned information the aim of the given investigation was to carry out comparative analysis of MMP-2,-9 expressions and their correlation with Mg\(^{2+}\) ions, glycosaminoglycans and glucuronidase activity in the blood of patients with congenital PMV.

**Materials and methods**

86 persons, 36 (41.9%) males and 50 (58.1%) females aged 15-25 (19.9±1.42) years with etiological signs of primary (idiopathic, congenital) PMV were included into popular investigation. The diagnosis was made of the basis of Kadurina’s classification (Kadurina and Gorbunova, 2008) and was confirmed by echocardiogram (EchoCG) 41 patient’s (47.7%) with regurgitation of the I degree (16 males and 25 females) that included into the 1\(^{\text{st}}\) group and 45 (52.3%) patients (20 males and 25 females) with the II degree into the 2\(^{\text{nd}}\) group. The person’s secondary PMV character, in careful examination of anamnestic data and the results of instrumental examinations as well as the patient with cardiovascular system diseases and rheumatism were not included into the investigations. The control group consisted of 20 relatively healthy persons aged 18.0±1.56 years without signs of EchoCG disturbance and those who voluntarily and orally agreed to undergo the examination. Patients of the basic and control group have been included in order to carry out investigation who gave the informed orally consent for performing of the investigation.
In all studied persons the blood from the ulnar vein was taken on an empty stomach in the morning in order to isolate blood serum and to determine: $\text{Mg}^{2+}$ mmol/l (on the atomic absorptive spectrofluorometer of AF-610-A, China); the level of total glucosamine glucans in mkml/l and hyaluronidase activity in mkml/l by spectrofluorometric method (on the spectrofluorometer F-96, China) using the modified method of P.N. Sharaeva et al. (1996); activity of matrix metalloproteases MMP-2 and MMP-9 and TIMP-1.

Markers were determined with the help of standard sets for direct enzyme-linked immunosorbent assay (ELISA), according to producers’ instruction. Measurements were carried out on the automatic universal reader AT-858 (China). Concentrations of the studied indexes in the blood serum were expressed in ng/ml. The received results were statically processed with employment of date bases of Microsoft Office Excel 2007 programmers the packet of BioStat software. The received results were expressed as $M\pm m$ with employment of Student’s t criterion and also simultaneously estimated correlative dependence among values of indexes according to Pearson. Differences $p<0.05$ are considered to be reliable.

The results and discussions

According to the results of the study the patients with PMV had increase of MMP-2 and MMP-9 and decrease of TIMP-1 in their blood. Simultaneously with the progress of the disease MMP-2 and MMP-9 expressions increase and TIMP-1 decreases in comparison with the date in the control group (Table 1).

Correlation balance of integral coefficient between MMP and TIMP-1 is impaired. It is estimated according to MMP-2+MMP-9 / TIMP-1 formula which is higher in patients with regurgitation of the 1st type than in the control group on 11.8% (p<0.05) and regurgitation of the 2nd type on 31.7% (p<0.01). Simultaneously in patients with PMV, decrease of $\text{Mg}^{2+}$ ions is noted; it was lower in the first group on 8 % (p>0.05) and on 16.5% (p<0.05) in the 2nd group in comparison with the data in the control group. On the background of $\text{Mg}^{2+}$ ions decrease in the blood, increase of proteolytic enzymes GAG and GN is noted. They were 4.5 (3.8%) (p<0.01) higher in the 1st group and 9.5 (12.2%) (p<0.05) higher in the 2nd group in comparison to the control group. Thus, in patients with I and II PMV regurgitation degree, decrease of $\text{Mg}^{2+}$ ions level is noted and it simultaneously causes the disturbance of collagen formation processes, intensification of synthesis of a typical low-molecular GAG, that passes into the blood stream. It can be suggested that intensified process of GAG going out of tissues surpasses their disintegration under the MMP-2 and MMP-9 and also GN action. It is known that GN characterizes total activity of B-glucuronidases and B-glucosaminidases and is activated due to blocking by antiglucuronidase that reflects the condition of protective mechanisms of the body. GN activation may result from decrease of TIMP-1 expression. In order to justify the importance of increasing MMP-2 and MMP-9 with TIMP-1, the activity of GAG and GBV, and reduction of Mg 2 in the pathogenesis of the disease we carried out

<table>
<thead>
<tr>
<th>Group</th>
<th>$\text{Mg}^{2+}$, mmol/l</th>
<th>GAG, mkml/l</th>
<th>Gn, mkml/l</th>
<th>MMP-2, ng/ml</th>
<th>MMP-9, ng/ml</th>
<th>TIMP-1, Ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st gr(n=41)</td>
<td>0.83±0.026</td>
<td>5.08±0.22</td>
<td>211.2±5.41</td>
<td>201.7±8.29</td>
<td>505.5±14.08</td>
<td>395.1±12.92</td>
</tr>
<tr>
<td>2nd Gr(n=45)</td>
<td>^0.76±0.021</td>
<td>^5.32±0.18</td>
<td>^222.3±5.8</td>
<td>^220.8±10.86</td>
<td>^547.6±10.12</td>
<td>^362.7±10.5</td>
</tr>
<tr>
<td>Control group (n=20)</td>
<td>0.91±0.051</td>
<td>4.86±0.204</td>
<td>203.5±9.05</td>
<td>190.5±8.9</td>
<td>485.6±21.7</td>
<td>420.8±20.03</td>
</tr>
</tbody>
</table>

Note: * - p<0.05 in comparison with control. ^ - p<0.05 in comparison with the 1st group.
Pearson correlation analysis (2) between these parameters. So the 1st group of patients showed a certain dependence between increase of MMP-2 and MMP-9 parameters and decrease of TIMP-1 index, Mg\(^{2+}\) (r=0.22-0.28, p>0.05) ions, increase of GAG and GN (r=0.24-0.31, p>0.05) activities in comparison with control data. Together with this inpatients of the 2nd group MMP-2 and MMP-9 expression had strict, strong, reverse correlation with the parameter of TIMP-1 (r=0.83-0.82, p<0.01) activity, and also in establishing connection with decrease of Mg\(^{2+}\) ions parameter (r=0.69-0.80, p<0.02 and p <0.01), and strong straight line with GAG (r=0.80-0.832, p<0.01) and GN (r=0.77-0.81, p<0.01) parameters. TIMP-1 expression in association with decrease of Mg\(^{2+}\) ions (r=0.75, p<0.01) level in the blood and in reverse process was connected with indexes of proteolytic enzymes GAG (r=-0.84, p<0.01) and GN (r=-0.86, p<0.01) activity. Thus, it may be suggested that the important course of increase of mitral regurgitation in patients with congenital PMV is MMP activation, including MMP-2 and MMP-9 as a result of reduction of TIMP-1 inhibitory effect, Mg\(^{2+}\) ions content, induction of proteolytic enzymes GAG and GN. Taking into account that in patients with PMV, MMP-2 and MMP-9 and as a result of decrease of inhibitory effect of TIMP-1, Mg\(^{2+}\) ions content which lead to the increase induction of proteolytic enzymes GAG and GN.

**Conclusion**

In patient with congenital PMV increase of MMP-2 and MMP-9 expression and decrease of TIMP-1 were revealed and their correlation with decrease of Mg\(^{2+}\) ions content, induction of GAG and GN. It was more marked in patients with the II degree of regurgitation in comparison with the 1st degree. Dynamics of changes of MMP-2 and MMP-9 and also TIMP-1 expression, depending on regurgitation degree in patients with PMV may serve as a method of estimation of progressive pathological process and prognosis of the disease.

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