LYSYL OXIDASE EXPRESSION IN AORTIC STENOSIS REGULATE THE DEGREE OF COLLAGEN CROSS-LINKING AND THE ELASTIC PROPERTIES OF THE LEFT VENTRICLE

F. Valencia Serrano1, B. López Salazar2, JJ Gómez Doblas3, J. Díez3, E. de Teresa Galván1

1 Hospital Virgen de la Victoria, Málaga. 2 Centre for Applied Medical Research, Pamplona. *Complejo Hospitalario Torrejón, Almería. Spain.

Purpose: We sought to analyze the expression of lysyl oxidase (LOX) enzyme, the degree of collagen cross-linking and its consequences in the myocardium of aortic stenosis (AS) patients.

Methods:

**Inclusion Criteria:**
- Aortic Valve Area < 1 cm²
- Mean Gradient > 40 mmHg
- Symptoms attributable to AS

**Exclusion Criteria:**
- More than mild to moderate MR or AR
- Renal or Hepatic Insufficiency
- Prior Myocardial Infarction

**COLLAGEN CROSS-LINKING**

- The degree of cross-linking was calculated as the ratio between the insoluble and the soluble forms of collagen. To distinguish between cross-linked (insoluble) and non-cross-linked (soluble) collagen a colorimetric procedure was employed:
  1. A fast green-staining was performed to identify and quantify total collagen.
  2. A Sirius-based assay was performed to obtain and quantify soluble collagen.

**LOX expression**

- Immunohistochimical and western blot analysis were applied to determine LOX expression using a mouse monoclonal antibody against LOX (R&D Systems).

**Collagen Volume Fraction**

- The fraction of myocardial volume occupied by fibrillar collagen (CVF) was determined by quantitative morphometry with an automated image analysis system in sections stained with collagen-specific Sirius Red.

- CVF was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas in all the fields analyzed in each section.

**Clinical Characteristics**

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Aortic Valve Area (cm²)</th>
<th>Mean Gradient (mmHg)</th>
<th>Medical Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71±1</td>
<td>0.64±0.04</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>10/24</td>
<td>54±2</td>
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<tr>
<td>Body Mass Index (kg/m²)</td>
<td>28±1</td>
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<td></td>
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<tr>
<td>High Blood Pressure</td>
<td>24</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>12</td>
<td>3</td>
<td></td>
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<tr>
<td>Coronary Artery Disease</td>
<td>12</td>
<td>12</td>
<td></td>
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<tr>
<td>Heart Failure</td>
<td>19</td>
<td>6</td>
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</table>

Categorical data are expressed as number of pts with the characteristic and continuous data as mean ± S.E.M.

**CONCLUSION:** LOX OVER-EXPRESSION IN THE MYOCARDIUM OF AS PATIENTS IS ASSOCIATED WITH WALL STRESS, DETERMINING THE DEGREE OF COLLAGEN CROSS-LINK, WHICH CAN AFFECT THE ELASTIC PROPERTIES OF THE LEFT VENTRICLE AND IS ASSOCIATED WITH HEART FAILURE. QUANTITATIVE AND QUALITATIVE COLLAGEN ALTERATIONS OBSERVED IN AS PATIENTS AND ITS CONSEQUENCES ARE NOT RELATED.