Arquivos Brasileiros de Cardiologia

REFERÊNCIA

MOURA, Edmilson Bastos de *et al*. Amplification of the genes that codify endothelin-1 and its receptors in rheumatic mitral valves. **Arquivos Brasileiros de Cardiologia**, São Paulo, v. 95, n. 1, p. 122-130, jul. 2010. DOI: https://doi.org/10.1590/S0066-782X2010005000059. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0066-782X2010001100018&lng=pt&nrm=iso. Acesso em: 04 dez. 2020.

Amplification of the Genes that Codify Endothelin-1 and its Receptors in Rheumatic Mitral Valves

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Abstract

Background: Cardiopathies are high prevalence conditions. Among them, rheumatic carditis is of high relevance in developing countries. Left cardiac chamber changes are associated to endothelial dysfunction and ET-1 levels increase. Pulmonary circulation is then affected, and not seldom leading to pulmonary hypertension (PH). However, the presence of ET-1 and its receptors in the mitral valve itself - promoting pulmonary vascular changes, with increased rheumatic valvular deformation - has not been discussed in the literature.

Objective: To determine the expression of endothelin gene and its receptors in rheumatic mitral valves through techniques of molecular genetics.

Methods: Twenty-seven patients submitted to mitral valve replacement had their valvular tissue examined to determine the presence of ET-1 genes and their A and B receptors. Histological and molecular analysis of the valves was performed (divided into M1, M2 and M3 fragments), with patients' clinical and epidemiological data collected. Patients were divided into 3 groups (mitral valvopathy, mitroaortic valvopathy, and reoperation patients).

Results: The study showed endothelin-1 gene expression in 40.7% specimens and A receptor in all samples; receptor gene B had lower expression (22.2%).

Conclusion: All patients showed A receptor gene expression. No statistically significant difference was observed in regard to condition severity, expressed according to functional class, and subgroups (mitral valvopathy, mitroaortic valvopathy, and reoperation patients). (Arq Bras Cardiol. 2010; [online]. ahead print, PP.0-0)

Key words: Myocarditis; cardiomyopathies; rheumatic diseases; endothelium; infection; rheumatic fever.

Introduction

Rheumatic fever (RF) is a rheumatic, inflammatory, recurring disease, mediated by autoimmune responses of the body to Lancefield Group A streptococcal infections (*Streptococcus pyogenes*). Some strains are called rheymatogenic due to distinctive virulence level in this subgroup - M-protein and hyaluronic acid-rich in its capsule - determining anti-phagocytic properties[1]. When submitted to average density analysis, rheumatic valves present larger measures, larger muscle and collagen content. As age advances, increased density with no significant valvular composition[2] can be observed.

Endothelin - a powerful vasoconstrictor (ten times more powerful than angiotensin II) - was described by Yanagisawa in 1988[3]. The relevance of endothelin, as well as of its receptors in the pathogenesis of a number of diseases - especially those where vasoconstriction and cellular overproliferation are involved - has been the focus of extensive research. Additionally, they play a significant part in collagen deposition and extracellular matrix[4]. They have multiple biological effects through their A and B receptors, and their tissue concentrations are more accurate reflection of endothelin system activation[4]. ET-1 may act as a link between vascular changes and collagen accelerated metabolism associated to fibrotic process under certain conditions[5]. Estimulated by friction forces, ET-1 synthesis and expression are increased. That physical factor - found in valvular dysfunctions under deficient coaptation between leaflets and likely to occur through increased friction - is associated to other chemical and endogenous factors, resulting in tissue composition changes. However, studies are being conducted to demonstrate the correlation between vasoconstricting effects and tissue fibrosis[5]. That pathological mechanism may be shown to be relevant in rheumatic valvopathy by associating ET-1 increased levels as found in the condition and valvular deformities.

A number of cardiovascular diseases have shown to include ET-1 in their pathogenesis. In the light of the arguments above, therapeutic applications may be expected from ET-1 receptors antagonists[5]. Through them, endothelin system actions may be reverted and translated into clinical benefit for quite a number of patients.

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Manuscript received November 11, 2008; revised manuscript received November 11, 2008; accepted January 29, 2009.

Methods

Twenty-seven samples of valves were collected at Carciovascular Surgery Services (*Instituto do Coração InCor-DF* and *Hospital de Base do DF*), and later submitted to histological and molecular analysis. The patients - adult males and females - were selected from those admitted with rheumatic mitral disease and surgical indication for prosthetic native valve replacement.

Study inclusion criteria were the following:

- Mitral valvopathy patients with rheumatic evidence on echocardiogram;
- Mitral disease patients - whether isolated or in association with another valvopathy (aortic, tricuspid);
- Surgery intervention indication based on selected Cardiovascular Surgery Units criteria, with no indication for percutaneous valvotomy or valve repair (commissurotomy/mitral valvoplasty):

Stenosis

- Moderate or severe (mitral valve area ≤ 1.5cm^2) and functional classification III or IV (NYHA), non-candidates to percutaneous valvotomy or valve repair;

Heart failure

- Symptomatic patients (NYHA II, III and IV), with normal left ventricular function (ejection fraction > 60% and LV endosystolic diameter < 45 mm);
- Symptomatic or asymptomatic patients, with mild left ventricular dysfunction (50 to 60% ejection fraction and 45 to 50 mm LV endosystolic diameter);
- Symptomatic or asymptomatic patients, with mild l left ventricular dysfunction (30 to 50% ejection fraction and 50 to 55 mm LV endosystolic diameter);
- Reoperation of mitral valve, provided mitral valve tissue was still native (that is to say, when the first surgery was for valve repair - (commissurotomy/mitral valvoplasty);
- Consent from patient or legal representative to participate in the study (Informed Consent having been signed).

Exclusion criteria included:

- Patients who were candidates for percutaneous valvotomy or valve repair surgery;
- Other cardiovascular diseases patients with surgery indication. Some examples are: obstructive coronary heart disease, LV aneurysm, congenital, cyanotic or acyanotic cardiac abnormalities;
- Reoperation on patients already submitted to mitral valve replacement (absence of native mitral valve tissue);
- No consent from patient or legal representative to participate in the study.

The patients were hospitalized and submitted to routine preoperative exams at each institution. After the patients were informed and consulted on their consent to participate in the study, they were given the Informed Consent Form and agreed to sign. Personal and epidemiological patient data were collected, as well as information on symptoms and functional classification (NYHA), echocardiography data (major mitral valvopathy, valvular area estimate, pulmonary systolic pressure estimate, effective regurgitant orifice area, regurgitant volume). Study Protocol was approved by the Research Ethics Committee at the School of Health Sciences, University of Brasilia - UnB (*Faculdade de Ciências da Saúde da UnB [Universidade de Brasília]*).

Immediately after excision, the valve segment that was obtained was fragmented as follows: When considering excised fragment extension, it always contained valve tissue from the free end to the area close to the mitral ring. That valvular tissue was then separated into 3 segments that were roughly the same size, and named M1, M2 and M3: the first one, close to the mitral ring; the third one, at the end, in contact with chordae tendineae; and the second one in intermediate position. Such segmentation was intended to differentiate the areas - which present macroscopic differences in rheumatic disease affection.

Histology

All excised, fragmented valves were formalin-fixed (neutral 10% solution - pH 7.0). They were later submitted to decalcification, paraffin-embedded, and microtome cutting (4 μc thick). Coloring was performed through hematoxylin and eosin procedure. Each segment was analyzed for fibrosis, calcification, ossification, vascular neoformation, and mononuclear infiltrate.

Total RNA extraction

Total RNA extraction from the 27 samples was performed through the use of Trizol™ (*Total RNA isolation Reagent* - Invitrogen™). Total RNA from the three different segments in the three mitral valve areas (M1, M2 and M3) was extracted separately. TRIZOL reagent was used (100μl).

The method consists in the maceration of approximately 100 μg of valvar tissue (previously frozen at - 80ºC), through the use of a manual motor (Cordless - Kontes) in laminary flow hood. Once homogenized, the tissue was submitted to repeated centrifugation, chloroform purification, isopropanol precipitation, and cleaned with 75% ethanol.

RNA quantification through spectrophotometry

4 μL RNA diluted in 196 μl of milliQ water. Total solution volume was 200 μl. The samples were placed in specific cuvettes and analyzed on the Spectrophotometer (UV-1601 - UV Visible Spectrophotometer - Shimadzu Corporation). Absorbance values found were analyzed following the formula: [RNA (μg/ml)] = 40 x A260 x dilution / 1000 (Maniattis). Purity was assessed through the ratio between absorbance values obtained at 260 nm and 280 nm (A260 / A280). Samples between 1.6 and 2.6 were considered viable.

cDNA

cDNA was obtained from the 27 patients under study through reverse transcriptase (RT). RNA - Trizol method extraction - was used and adjusted to a final concentration at 1 $\mu g/\mu l$ through simple rule of three. It was followed by recommended procedure for Promega ImProm-II™ Reverse Transcriptase kit. OligodT (dT_{18}) oligonucleotide concentration was 0.5 μg.

DNA amplification through CRP

From the cDNAs, CRP reactions were performed to detect gene expressions of interest in the valvular fragments of patients under study. Specific primers were then created for ET-1 (sequence NM 001955 gi110624717 - NCBI), ET_A (sequence NM 001957 gi4503464 - NCBI) and ET_B (sequence NM 000115 gi4557546 - NCBI). Gene GAPDH (BC029640) was used as sample control. All primers were diluted to 10mM concentration. Invitrogen reagents were used for the reaction, when 1.25 U *Taq* DNA polymerase (5 U/μl), 0.25 μM from each primer, and dNTPs concentrated at 0.1 mM each were used. The programs used for the amplification were defined based on primers manufacturer's Analysis Certificate (Invitrogen Brasil LTDA.). Reaction final volume was 10 μl. Reactions were performed in triplicate. All samples under analysis were verified in 1% agarose gel.

DNA analysis through agarose gel electrophoresis

After amplifications, 5 μl from each sample was analyzed through 1% agarose gel electrophoresis, had ethidium bromate added (5 mg/ml) and sample buffer (bromophenol blue) 6x.

Statistical analysis

Data statistical analysis was performed through Fisher's Exact Test. This is a statistical test where p is calculated as if marginal totals were fixed; below 0.05 was considered significant.

Results

Twenty-seven patients submitted to surgical treatment between March, 2006 and September, 2007, following previously determined inclusion and exclusion criteria. Two of them were operated on at the *Hospital de Base*, District Capital, and 25 at District Capital Heart Institute (Instituto do Coração do DF). All patients gave their consent to participate in the study. Fifteen patients were females, and 12, males. Mean age was 46.2 (age range 16-67 years; SD: 12.6 - Table 1). From the total sample, only 10 patients (3, 4, 7, 9, 11, 18, 20, 22, 24, 25 = 37%) could inform on condition time length - which ranged from 4 months to 45 years (mean time 12 years). No death was reported transoperatively, postoperatively or at hospital.

From the individual data collected, patients were observed to be in the four functional classifications (FC) (NYHA), as follows: One patient in Class I (patient 21 - 3.7% of the population); 14 patients in Class II (patients 1, 2, 3, 7, 9, 10, 11, 16, 17, 18, 19, 20, 24 and 26 - 51.8%); 11 patients in Class III (patients 4, 5, 6, 8, 13, 14, 15, 22, 23, 25 and 27 - 40.7%), and 1 patient in Class IV (patient 12 - 3.7%). As for valvular lesion prevalence, 19 patients reported double lesion (70.3%); 5 patients reported pure valvular insufficiency (18.5%); and the 3 remaining patients (11.2%), pure valvular stenosis (Table 1).

Echocardiographic data obtained were valvular area, mitral transvalvular gradient, and pulmonary artery systolic pressure (in mitral stenosis cases), and regurgitant volume and effective regurgitant orifice. The findings showed mean valvular area to be 1.1 cm^2 (SD: 5.3; 0.6-1.9 cm^2 - data from all 22 patients), mean transvalvular gradient =14.8 mmHg (SD: 6.9; 6-32 mmHg - data from 20 out of the 22 patients = 90.9%); mean pulmonary artery systolic pressure = 55.9 mmHg (SD: 16.4; 21-100 mmHg - data from 21 out of the 22 patients = 95.4%); mean regurgitant volume = 88 mmHg (SD: 61.3; 58-209 ml - data from 6 out of the 24 patients = 25%); mean effective regurgitant orifice = 0.47 cm^2 (SD: 0.26; 0.27-1 - data from 6 our of 24 patients = 25% - Table 1).

Most commonly found clinical manifestations were dyspnea (25 out of the 27 patients = 92.5%); chest pain (9 patients = 33.3%); nocturnal paroxysmal dyspnea (7 patients = 25.9%); edema in the lower limbs (3 patients =11.1%), and orthopnea (3 patients = 11.1%). Two patients (7.4%) presented palpitations, 1 patient (3.2%) presented tachypnea and hemoptoic sputum. One patient declared to be asymptomatic at hospital (Tables 2, 3 and 4).

As for surgical approach, 5 patients (18.5% - patients 2, 4, 8, 22 and 23) were submitted to reoperation, whereas the remaining (22 patients = 81.5%) had surgical intervention for the first time. From those reoperated on, mean time from first surgery was 12.2 years (SD: 6.3; patient 22 = 5 years, patient 2 = 9 years; patient 23 = 10 years; patient 8 = 16 years; patient 4 = 21 years).

RNA quantification showed absorbance ratio within the range seen as appropriate in 91.3% of patients (21 out of 23 samples) in M1; in 87.5% of patients in M2 (21 out of 24 samples); and in 92% the samples in M3 (23 out of 25 samples). The samples were submitted to cDNA.

Agarose gel analysis was performed in 21 out of the 27 collected samples (77.8%). ET-1 gene was shown in 40.7% (11 out of the 27 samples - patients 1, 2, 3, 8, 9, 12, 14, 22, 23, 24, 26). A receptor (ETrA) was shown in all 27 samples under investigation (100%). B receptor corresponding gene (ETrB) was identified in 6 samples (28.6% - patients 1, 9, 13, 21, 22 and 26).

Histology

After valvular fragments were collected, twelve were paraffin-embedded, with coloring performed through hematoxylin and eosin procedure. Afterwards, each fragment was analyzed for fibrosis, calcification, ossification, vascular neoformation, and mononuclear infiltrate. Plates were then done and identified as M1, M2 and M3, depending on material availability, which was prioritized for RNA extraction stage and subsequent stages.

High level of fibrocytes, dense connective tissue, collagen fibers (eosinophilic) and extracellular fundamental substance could be observed. The tissue is roughly avascular. No Anitschkow myocytes or Aschoff nodules were found.

Table 1 - Patient's echocardiographic data

Patient	Gender	Age	FC (NYHA)	Valvular dysfunction	Valvular area (PHT)	g	PSAP	R.V.	E.R.O.
1	M	43	2	D	1	17	55	-	-
2	F	54	2	I	1,9	7	56	83	0.5
3	M	16	2	I	-	-	-	-	0.27
4	F	51	3	D	1,1	10	40	-	-
5	F	49	3	D	1,9	8	51	58	-
6	F	22	3	D	0,6	13	60	-	-
7	M	35	2	D	1,4	12	-	-	-
8	F	67	3	D	1	14	56	209	1
9	M	62	2	D	-	9	70	-	-
10	F	50	2	D	0,9	19	-	-	-
11	F	47	2	D	1	-	51	-	-
12	M	62	4	I	-	-	63	-	-
13	F	63	3	D	0,9	17	71	-	-
14	F	44	3	D	0,9	14	40	-	-
15	M	39	3	D	1,1	9	21	-	-
16	F	51	2	D	1	18	55	76	0.4
17	F	59	2	D	1	32	75	-	-
18	M	46	2	D	1,4	12	100	-	-
19	F	50	2	S	0,8	22	74	-	-
20	M	38	2	I	-	-	35	68	0.4
21	M	52	1	I	3,1	5	49	36	0.3
22	F	26	3	D	1,4	-	46	-	-
23	M	49	3	D	1	11	55	-	-
24	F	52	2	E	0,9	12	44	-	-
25	F	39	3	D	0,8	28	64	-	-
26	M	54	2	D	-	-	-	-	-
27	M	30	3	E	1	23	-	-	-

FC (NYHA) - functional class (New York Heart Association); G.T.M. - transvalvular mean gradient; R.V. - regurgitant volume; E.R.O. - effective regurgitant orifice.

The M3 fragments showed high cellularity inflammatory process, tissue vascularization - with permeating capillaries (and higher tissue vascularization as compared to the other areas), collagen transformation or neoformation (collagen hialinization). Fibroblasts, lymphocytes, and dystrophic calcification areas were observed (deposit tissue necrosis). The findings in M2 plates were similar: healing areas, small inflammation foci, and cells more drawn apart. Ossification could also be observed. M1 segment histologic analysis showed the same characteristics as described earlier, except for the absence of calcification or ossification. The individual histologic findings were the following:

- Fibrosis in all patients on M3 sample (2, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 22); in 10 patients in sample M2 and M1 (2, 5, 8, 9, 10, 11, 12, 14, 15, 22) - 83.3% of total number of patients under analysis.
- Vascular neoformation in 9 patients (2, 5, 6, 8, 9, 11, 14, 15, 22) - 75% of the total number of patients under analysis; in two M1 samples (22.2%); in four M2 samples (44.4%), in five M3 samples (55.5%) and in two patients (11 and 15 - 22.2%) it could be observed in two fragments concurrently (M1/M3 and M2/M3, respectively).
- Mononuclear infiltrate was found in 6 patients (2, 5, 6, 8, 9, 22) - 50% of the total number of patients under analysis; in one M1 sample (16.6%), in three M2 samples (50%), in two M3 samples (33.3%); and in no patient in two or more fragments concurrently.
- Calcification was observed in 3 patients (9, 13, 22) - 25% of the total number of patients under analysis; not observed in M1 samples, found in one M2 sample (33.3%), in two M3 samples (66.6%). Concurrent calcification in two or more fragments was not observed in any of the patients.

Table 2 - Clinical data and individual molecular analysis of patients with mitral valvular impairment only

Patient	FC (NYHA)	Severity	Symptoms	Fragment	ET-1	ETrA	ETrB1
1	2	Moderate	1	M3	+	+	+
5	3	Severe:	1,2,3	M2		+	
6	3	Severe:	1,3,5	M3		+	
9	2	Severe:	1,2,3,5,6	M1	+	+	+
10	2	Severe:	1	M2		+	
12	4	Severe:	1,3,5	M1	+	+	
13	3	Severe:	1	M3		+	+
15	3	Severe:	1,2	M2		+	
16	2	Severe:	1,2	M2		+	
17	2	Moderate	1	M3		+	
18	2	Severe:	1,8	M3		+	
19	2	Severe:	1,7	M3		+	
24	2	Severe:	1,3	M1	+	+	
25	3	Severe:	1	M3		+	
26	2	Moderate	1	M2	+	+	+

Symptoms: dyspnea (1); chest pain (2); nocturnal paroxysmal dyspnea (3); edema in lower limbs (4); orthopnea (5); hemoptoic (6); palpitations (7); tachypnea (8).

Table 3 - Clinical data and individual molecular analysis of patients with mitral valvular impairment only

Patient	FC (NYHA)	Severity	Symptoms	Fragment	ET-1	ETrA	ETrB1
3	2	Moderate	2	M2	+	+	
7	2	Severe:	1	M3		+	
11	2	Severe:	1,2	M1		+	
14	3	Severe:	1,2,4	M2	+	+	
20	2	Severe:	1,2,7	M2		+	
21	1	Severe:	-	M3		+	+
27	3	Severe:	1	M3		+	

Table 4 - Clinical data and individual molecular analysis of patients submitted to mitral reoperation

Patient	FC (NYHA)	Severity	Symptoms	Fragment	ET-1	ETrA	ETrB1
2	2	Severe:	1,3,4	M3	+	+	
4	3	Moderate	1	M1		+	
8	3	Severe:	1	M3	+	+	
22	3	Severe:	1,2	M1	+	+	+
23	3	Severe:	1,3,4	M1	+	+	

- Ossification was observed in only one sample (M2) - patient 9 - 8.3% of the total number of patients under analysis.

Discussion

The study showed endothelin-1 gene expression in 40.7% specimens (11 of the 27 valves) and A receptor in all samples of rheumatic valves for the population described; receptor gene B showed lower expression (6 of the 27 samples - 22.2%).

Patients were selected based on the indication for valvular surgery correction[6]. The information from medical records - many times quite relevant - were irreversibly lost, and therefore could not be taken into consideration in the present study.

Individuals who had been submitted to previous valvuloplasty - patients with valvular fragments affected by

artificially increased inflammatory reaction from previous surgery manipulation - were not excluded due to their relevance in the group - to be compared with patients who were being submitted to the first surgical intervention. The number of patients submitted to reoperation corresponded to 18.5% of all cases (5 patients) - a significant percentage from the total number of cases.

As the authors could observe (based on Table 1 and 4) reoperation patients in this series were mostly females. Among them (80% - 4 females), 75% (3 out of 4 females) were menopausal women (patients 2, 4 and 8). Although the figures are not expressive, the differential hormonal condition of those patients may determine changes in the expression of peptide-codifying genes in the endothelin system.

The authors have chosen to subdivide the valve into four different regions, following descriptions that show valvular heterogeneity when longitudinal sectioning is considered (from free end up to its insertion in the mitral ring). In that sense, the authors tried to show evidence of possible differences between segments (M1, M2, and M3) while identifying ET-1 genes and A and B receptors. ET-1 is known to play a key regulating function in collagen deposition in the tissues[7]. It is yet to be defined whether collagen tissue composition increase somehow affects the presence or the concentration level of ET-1 and its receptors[8]. However, the lack of samples did not allow enough valvular tissue to be collected for histological and molecular analysis, or even the analysis of the three segments of interest. Another difficulty faced by the authors - when fragments were collected from the three areas - was to find low cellularization samples, from which little nuclear content could be extracted.

The following stage - RNA extraction from the samples - had the purpose to show the expression of one gene. The process required minimal loss and degradation of the fragment (many times extremely small). The tissue under study was poorly cellularized, collagen and elastin-rich, and poor in mesenchymal cells (such as cardiac myofibroblasts and cardiac e myocytes). That was a key influence to obtain RNA, since RNA is obtained from the latter. The resulting RNA sample was invariably scarce.

RNA quantification was then performed through spectrophotometry. The variation considered adequate ranged from 1.6 and 2.6 of ratio mentioned. Table 6 shows that the results obtained from such reading inform on adequate values for the great majority of the samples, thus indicating satisfactory purity level.

The presence of ETrA in the samples is an interesting, expected result, since in addition of being the predominant receptor in cardiac myocytes[9], this receptor is associated to exuberant inflammatory processes in rheumatic diseases exudative phase, and also present in the proliferative stage[10]. Although study subjects were at later stages - when only healing sequelae from earlier processes remain[10] - the authors believe that residual cellular receptors may have remained even after the defervescence of the primary inflammatory activity.

B receptors (ETrB) were found in few samples of the series, as seen in Tables 2, 3, and 4. As discussed earlier, those peptides can be found in the endothelial and epithelial cells that are responsible for the modulation of vascular resistance and natriuresis, among others[11]. Additionally, those receptors are characterized by fast desensibilization and internalization, whose results in tissue concentration have not been extensively investigated[12]. The authors believe that rheumatic valvular disease associated to other individual characteristics may be determinant for the manifestation of ETrB in mitral valve tissue. Such statement will require specific research.

The authors believe the major study limitation is due to the absence of a control group that would be free of rheumatic disease or any other kind of disease, and from which similar information could be obtained on the presence

Table 5 - Patient's individual histological data

Patient	Histology														
	Fibrosis			Vascular neoformation			Mononuclear infiltrate			Calcification			Ossification		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
2	x	x	X		X			x							
5	x	x	X		X			x							
6			X			X			x						
8	x	x	X			X			x						
9	x	x	X		X			x			x			x	
10	x	x	X												
11	x	x	X	X		X									
12	x	x	X												
13			X									x			
14	x	x	X			X									
15	x	x	X		X	X									
22	x	x	x	X			x					x			

Table 6 - Data on total RNA extraction from samples. UnB-FM. 2006-2008

Patients	M1						M2						M3					
	Quantific.	260	280	Ratio	[ ] µg/µl	p/ [ ] 1µg/µl	Quantific.	260	280	Ratio	[ ] µg/µl	p/ [ ] 1µg/µl	Quantific.	260	280	Ratio	[ ] µg/µl	p/ [ ] 1µg/µl
1	YES	0.047	0.014	3.3	0.094	10.6	YES	0.074	0.033	2.2	0.148	6.8	YES	0.231	0.130	1.8	0.462	2.2
2	YES	0.066	0.041	1.6	0.132	7.6	YES	0.002	-0.010	-0.2	0.004	250.0	YES	0.139	0.068	2.0	0.278	3.6
3	YES	0.037	0.026	1.4	0.074	13.5	YES	0.199	0.116	1.7	0.398	2.5	YES	0.104	0.045	2.3	0.208	4.8
4	YES	0.105	0.048	2.2	0.210	4.8	YES	0.082	0.041	2.0	0.164	6.1	YES (-)	0.028	0.008	3.5	0.056	17.9
5	YES	0.034	0.02	1.7	0.068	14.7	YES	0.105	0.066	1.6	0.210	4.8	YES	0.097	0.059	1.6	0.194	5.2
6	YES	0.072	0.036	2.0	0.144	6.9	YES	0.055	0.025	2.2	0.110	9.1	YES	0.087	0.038	2.3	0.174	5.7
7	YES	0.135	0.066	2.0	0.270	3.7	YES	0.017	0.006	2.8	0.034	29.4	YES	0.136	0.079	1.7	0.272	3.7
8	YES	0.077	0.040	1.9	0.154	6.5	YES	0.038	0.016	2.4	0.076	13.2	YES	0.131	0.070	1.9	0.262	3.8
9	YES	0.131	0.076	1.7	0.262	3.8	YES	0.052	0.020	2.6	0.104	9.6	YES	0.058	0.027	2.0	0.116	8.6
10	YES	0.116	0.051	2.3	0.232	4.3	YES	0.134	0.074	1.8	0.268	3.7	YES	0.114	0.051	2.2	0.228	4.4
11	YES	0.271	0.161	1.7	0.542	1.8	YES	0.503	0.289	1.7	1.006	1.0	YES	0.101	0.042	2.4	0.202	5.0
12	YES	0.378	0.199	1.9	0.756	1.3	YES	0.074	0.022	3.4	0.148	6.8	YES	0.101	0.040	2.5	0.202	5.0
13	YES	0.401	0.212	1.9	0.802	1.2	YES	0.266	0.151	1.8	0.532	1.9	YES	0.281	0.140	2.0	0.562	1.8
14	YES	0.081	0.040	2.0	0.162	6.2	YES	0.088	0.040	2.2	0.176	5.7	YES	0.113	0.071	1.6	0.226	4.4
15	YES	0.048	0.027	1.8	0.096	10.4	YES	0.137	0.072	1.9	0.274	3.6	YES	0.078	0.042	1.9	0.156	6.4
16	YES	0.079	0.039	2.0	0.158	6.3	YES	0.236	0.137	1.7	0.472	2.1	YES	0.109	0.061	1.8	0.218	4.6
17	YES	0.103	0.056	1.8	0.206	4.9	YES	0.227	0.131	1.7	0.454	2.2	YES	0.165	0.099	1.7	0.330	3.0
18	YES	0.113	0.061	1.9	0.226	4.4	YES	0.079	0.044	1.8	0.158	6.3	YES	0.287	0.155	1.9	0.574	1.7
19	YES	-	-	-	-	-	YES	-	-	-	-	-	YES	0.090	0.049	1.8	0.180	5.6
20	YES	0.206	0.121	1.7	0.412	2.4	YES	0.135	0.082	1.6	0.270	3.7	YES	0.313	0.191	1.6	0.626	1.6
21	YES	0.025	0.004	6.25	0.050	20.0	YES	0.120	0.056	2.1	0.240	4.2	YES	0.030	0.001	30	0.06	16.7
22	YES	-	-	-	-	-	YES	-	-	-	-	-	YES	0.080	0.037	2.2	0.160	6.3
23	YES	0.299	0.158	1.9	0.598	1.7	YES	0.031	0.016	1.9	0.062	16.1	YES	0.087	0.064	1.4	0.174	5.7
24	YES	0.488	0.297	1.6	0.976	1.0	YES	0.06	0.029	2.0	0.120	8.3	YES	0.156	0.081	1.9	0.300	3.3
25	YES	0.269	0.156	1.7	0.538	1.9	YES	0.04	0.028	1.4	0.080	12.5	YES	0.059	0.037	1.6	0.118	8.5
26	YES	0.062	0.024	2.6	0.124	8.1	YES	0.042	0.016	2.6	0.084	11.9	YES	0.074	0.037	2.0	0.148	6.8
27	YES	0.328	0.168	2.0	0.656	1.5	YES	0.419	0.236	1.8	0.838	1.2	YES	0.302	0.172	1.8	0.604	1.7

of the peptides that are the object of study. However, such specimens would only be obtained if from normal - and still feasible - valvular tissue.

Statistical analysis was performed through Fisher's Exact Test. The test was used to assess the significance of the association between the contingency tables. This is a statistical test used in the categorical data analyses when sample size is small. The test showed a trend - male patients in the populational sample showed higher probability to present ET-1, in addition to the strong trend towards the association of that gene and the lower limbs edema variables ($p = 0.056$) and nocturnal paroxysmal dyspnea ($p = 0.084$). However, whether due to the small size sample or *p* value, such correlation cannot be confirmed.

The search for the cure of rheumatic valvular cardiopathy through pharmacological agents rather than valvular replacement or surgical valvuloplasty still requires further research, and permeates a number of public health sectors. Streptococcal outbursts control through sanitary actions would be a good start. Early diagnosis to determine low or no involvement of valvular and subvalvular apparatus impairment would allow the management of countless individuals in a less costly fashion. The identification of valvular cardiopathy patients under risk through regular outpatient unit follow-up would refer them to invasive treatment before complications arise.

The combination of proper strategies in health policies and medical research in basic and clinical areas may help find options to current therapies. Against such scenario, the endothelin system and its antagonists may help by limiting valvular inflammatory reaction, modulating vasoreactivity, and changing the pathophysiologic and clinical progress of rheumatic valvulopathy.

Conclusions

Although no statistical significance was found, there is a trend towards the presence of ET-1 in male patients; All patients - irrespective of individual characteristics (gender, age range, mitral valve changes) - expressed A receptor gene.

Out of reoperation patient, 4 were menopaused women, one was of childbearing age and expressed the three genes under study.

No statistic difference was shown as to disease severity expressed in functional class and subgroups under study (mitral valve cardiopathy, mitro-aortic, and reoperation patients).

No statistic difference was shown as to the expression of ET`1 genes and their receptors among the subgroups under study (mitral valve cardiopathy, mitro-aortic, and reoperation patients).

The population under study showed higher presence of ET-1 among patients with edema in lower limbs and nocturnal paroxysmal dyspnea. Among those, females showed higher numbers.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Edmilson Bastos de Moura, from *Universidade de Brasília - UnB*.

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