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Article

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Abstract: Infective endocarditis (IE) results from infections by gram-positive bacteria, and, in this condition, the redox homeostasis is lost by overproduction of H_2O_2, leading to overstimulation of the immune system and up- regulation of the production of proinflammatory cytokines. The aim of this preliminary study of three cases with IE, was to evaluate the levels of oxidative biomarkers and of the enzymatic and non-enzymatic antioxidant systems, in subjects with IE. This is a preliminary retrospective analytical study of cases that had undergone aortic valve replacement surgery that was complicated by IE. We determined malondialdehyde (MDA), total antioxidant capacity (TAC), carbonyl groups, glutathione (GSH), thiols and the nitrate/nitrite ratio (NO_3^-/NO_2^-) in homogenized tissue of cardiac valves. We also measured the activities of GST, GPx, GR and TrxR. The super oxide dismutase (SOD) isoforms and peroxidase activities were determined using native gels. There were increases in the activities of antioxidant enzymes such as GST, SOD isoforms and peroxidases (p=0.01) and decreases in oxidative stress markers such as GSH, TAC, (p=0.01); however, MDA was increased (p=0.01). The results suggest that loss of the intricate redox homeostasis system in the aortic valve of patients with IE may favor the bacterial process and cause damage probably leading to a fatal outcome.

Keywords: infective endocarditis; oxidative stress; cardiac valve; antioxidant enzymes; redox homeostasis

1. Introduction

Gram-positive bacteria such as *Streptococcus* and *Staphylococcus*, are responsible for infective endocarditis (IE). These bacteria constitute 75% of the isolated microorganisms in this condition, but other species have also been found such as *Escherichia coli* and *Pseudomonas aeruginosa* among others. [1] The intracardiac site most commonly compromised is the aortic valve. The clinical characteristics present in the patients are fever, sepsis, septic shock and heart failure. The damage to the heart results from the formation of vegetations composed of fibrin and platelets [2]. Pharmacological treatment may include aminopenicillins, cephalosporins, and penicillin [3].

The gram-positive bacteria contain lipopolysaccharides (LPS) in their cell wall which are responsible for the overexpression of the host´s immune system, the induction of a cascade of proinflammation caused by cytokines and the adhesion to epithelial cells. These bacteria also have

powerful toxic effects [4]. The overstimulation of the immune system is mainly due to activation of macrophages, monocytes and neutrophils and there is increased secretion of proinflammatory cytokine such as interleukins (IL)-1, -6 and -8 which can, in turn, upset the redox homeostasis in the body. This homeostasis includes the enzymatic and non-enzymatic antioxidant system [5]. The lost redox homeostasis in IE is characterized by an increase of the super oxide anion (O_2^-) mainly derived from NADPH oxidase and mitochondrial dysfunction. This anion contributes to increase other oxygen reactive species (ROS) such as hydrogen peroxide (H_2O_2) [6]. ROS may also lead to the production of hydroxyl radicals (OH^-) that induce peroxidative damage to proteins, lipids, carbohydrates and nucleic acids in cardiomyocytes. This is due to the large amount of iron present in cardiomyocytes which is involved in the Fenton and Haber–Weiss reactions in presence of the OH–. In this sense, there is an association between generation of myocardial ROS and lipid peroxidation (LPO), and contractile dysfunction of the left ventricle [7]. Therefore, in this oxidative background an alteration of the antioxidant systems may be expected, but so far, the degree of participation of each particular antioxidant system remains unknown.

On the other hand, clinical management of IE, often requires of a surgical intervention or highly complex invasive procedures that are associated with several diagnostic and therapeutic dilemmas. These dilemmas are due to lack of experience of the clinicians and to the lack of published data and guidelines on the appropriate treatment [8]. Therefore, the mortality rates from this condition are high, and this constitutes a challenge in need of surveillance proposals. The existence of therapeutic guidelines for the treatment may be improve by implementing preventive and therapeutic management [9]. Therefore, the research of the role played by the deregulation of the redox homeostasis within the infectious process is relevant, regardless of the type of triggering agent of the disease, because the understanding of how redox homeostasis is compromised and its association with the process of bacterial infection could help induce a better outcome for patients. It would therefore be of importance to propose an adjuvant therapy that could contribute to re- establish the redox homeostasis. Therefore, the aim this preliminary study in three in subjects with IE, was to evaluate the levels of OS markers, the enzymatic and non-enzymatic antioxidant systems in native or prosthetic valves that required aortic valve replacement (AVR) or mitral valve. We also compare the results with those obtained from three subjects undergoing AVR without endocarditis. The results could highlight the need for future studies in a larger number of patients.

2. Materials and Methods

2.1. Recruited Patients with IE and Control Subjects

We performed a preliminary analytical retrospective review of the medical records in a list of patients diagnosed according to the modified Duke/ESC 2015 clinical criteria as patients with IE (protocol with identifier code CI-035-2024) [10]. The three selected cases with IE had been discussed by a team of expert surgeons, interventionists and clinicians who had agreed that they required a surgical or intervention by Transcatheter aortic valve implantation (TAVI) and the patients had given their informed consent for the intervention prior to surgery. Patients had also agreed that tissues could be used for microbiological and histopathological studies. In summary, the cases had had a clinically documented diagnosis which was supported by evidence, positive bacterial culture and imaging that have IE. Aortic and valve tissue were obtained during the valvuloplasty surgery due to the presence of endocarditis. Samples were obtained from the region proximal to the lesion by vegetation which could be present in different segments, and samples of each tissue were sectioned into two portions, placed in tubes under liquid nitrogen and frozen at -70 °C until studied. The control subjects were enrollment by the different disease in the surgery by AVR St. Jude with mechanical prosthesis; first case, surgery AVR; second case and mechanical AVR St. Jude; thirty cases. These patients were selected because of the valve replacement they underwent in the same way as the subjects with IE, although they are not healthy subjects and it is expected that they also present alterations in redox homeostasis, but here the difference lies in the IE present in the patients who did have the bacterial infection. Samples were obtained from the region proximal of the aortic valve

replacement, the samples of each tissue were sectioned into two portions, placed in tubes under liquid nitrogen and frozen at -70 °C until studied.

2.2. Echocardiographic Study

Transesophageal echocardiography was performed using a X7-2t, transducer 2-7mHz (Phillips). All patients underwent a comprehensive study for high clinical suspicion of endocarditis under sedation.

2.3. Cardiac Valve Homogenization

The segment from the aortic valve was homogenized under liquid nitrogen after adding KH_2PO_4 (2 mL) 0.05 mM, pH 7.3 in presence of 20 µL antiproteases inhibitors (1 mM PMSF, 2 μM pepstatin A, 2 μM leupeptin, and 0.1% aprotinin), and the preparation was kept on ice. The Lowry method was utilized to determine the protein concentration in the homogenates [11]. All assays on biochemical variables (except for those in the native gels and western blot analysis) were made in duplicate.

2.4. Determination of the Malondialdehyde

The malondialdehyde (MDA) level was read spectrophotometrically at 532 nm. A quantity of 100 µg of homogenized tissue from the aortic valve of the cases with IE and CS were used for this determination. Methanol with BHT at 4% (100 µL) and KH_2PO_4 buffer pH 7.4 (1 mL) was added to the sample and then it was incubated at 37 °C for 30 min after 2-thiobarbituric acid at 0.8 M (1.5 mL) was added. It was then incubated at 90 °C for 1 h. Then, KCl at 5% (1mL) and n-butanol (4 mL) were added. The sample was shaken for 30 s, and centrifuged at 4000 rpm (2 min). The butanol phase was extracted, and the absorbance was measured [12].

2.5. Evaluation of Total Antioxidant Capacity

The total antioxidant capacity (TAC) was detected spectrophotometrically at 593 nm in 100 µg of homogenized tissue of the aortic valve of the IE and CS cases in presence of a mixture that contained $C_2H_3O_2$ at 300 mM, $FeCl_3 \cdot 6H_2O$ at 20 mM, 2,4,6-tris-2-pyridyl-s-triazine at 10 mM, HCl at 40 mM, at pH 3.6 (1.5 mL, at ratio of 10:1:1 v/v,). The samples were incubated at 37 °C for 15 min and the absorbance was measured [13].

2.6. Carbonylation

To evaluate the carbonyl groups, 100 µg of homogenized tissue of the aortic valve were mixed with HCl 2.5 M (500 µL) and in parallel, another sample was mixed with 2,4-dinitrophenylhydrazine (500 µL) and incubated in the dark at room temperature for one hour. At the end of the incubation, $C_2HCl_3O_2$ at 20% was added (500 µL) and the sample was centrifuged at 15,000× g for 5 min. The button was recovered and washed two times by adding $C_2H_5OH/C_4H_8O_2$ (1 mL), then incubated for 10 min and centrifuged at 15,000× g for 10 min. Finally, CH_6ClN_3 at 6 M in KH_2PO_4 at 20 mM and pH 2.3 (1 mL) were added and the mixture was incubated at 37 °C for 30 min. The absorbance was read spectrophotometrically at 370 nm [12].

2.7. GSH and thiols.

For the determination of GSH, 100 µg of homogenized tissue of the aortic valve were used and this molecule was detected spectrophotometrically at 412 nm, according to Ellman's method. The thiol groups were read spectrophotometrically at 415 nm and 50 µg of homogenized tissue of the cardiac valve of IE and CS was used according to the Erel and Neselioglu's method [14].

2.8. NO_3^-/NO_2^- Ratio Determination.

To evaluate the NO_3^-/NO_2^- ratio, 100 µg of homogenized tissue of the aortic valve of IE and CS were previously deproteinized with 0.5 N, NaOH and 10%, $ZnSO_4$ (100 µL). Then, 10 µL of the

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cytochrome c reductase (NADPH) (Sigma Aldrich Cat# 24479) were added and the sample was incubated for 30 min at 37 °C. After the incubation period, sulfanilamide 1% and N-naphthyl-ethyldiamine 0.1% (200 µL respectively) were added and the total volume was adjusted with water distilled to 1 mL. The absorbance was measured at 540 [15].

2.9. Determinations of Antioxidant Enzymes That Employ GSH

To evaluate the activities of GST, GPx, GR and TrxR, 100 µg of homogenized tissue of the aortic valve of IE and CS, were employed [12]. The GR activity was expressed as µmol of reduced GSSG/min/mg of protein, with an extinction coefficient of 6220 M^{-1} cm^{-1}. The GST activity was expressed as units of GS-TNB mol/min/mg of protein, with an extinction coefficient of 14,150 M^{-1} cm^{-1}. The GPx activity was expressed as nmol of NADPH oxidized/min/mg of protein, with an extinction coefficient of 6220 M^{-1} cm^{-1} at 340 nm for NADPH. The samples were incubated and monitored at 340 nm for 6 min at 37 °C. For the TrxR activity, the sample was incubated and monitored at 412 nm for 6 min at 37 °C. The TrxR activity was expressed as TNB nmol/min/mL of the serum, with an extinction coefficient of 13,600 M^{-1} cm^{-1}.

2.1. Determinations of Super Oxide Dismutase Isoforms and Peroxidases Activities

The activities of super oxide dismutase (SOD) isoforms and peroxidase were determined through non-denaturing gel electrophoresis [12]. 25 µg of homogenized tissue of the cardiac valve of IE and CS were applied directly to non-denaturing 10% polyacrylamide gels. The electrophoresis was carried out at 120 volts for 4 h. For SOD isoforms activities, the gel was incubated with nitro blue tetrazolium at 2.45 mM for 20 min, then incubated with buffer of the KH_2PO_4 at 36 mM, EDTA at 28 mM, and riboflavin at 28 Mm pH 7.8 (20 mL) and exposed for 10 min to UV light. Purified SOD from bovine erythrocytes with a specific activity of 112 U/mg of protein (Sigma-Aldrich, St. Louis, MO, USA) was used as positive control for calculating the activity of these enzymes. For the peroxidase activity, the gel was washed with distilled water three times, for 5 min, after being incubated with 3 mg/mL 3,3,5,5-tetramethylbenzidine dissolved in CH_3-OH/CH_3COOH/H_2O (1:1:1 *v/v*) with H_2O_2 (300 µL) total volume 20 mL, for 10 min. A quantity of 35 µL of horseradish peroxidase was loaded to a final concentration of 178.5 µg as a standard. The activities in the SOD isoforms and peroxidase gels were analyzed using densitometry with a Kodak Image® 3.5 system.

2.11. Statistical Analysis

Continuous variables are expressed as median with minimum and maximum values. Categorical variables, such as frequencies and percentages, are reported. Normality distribution was evaluated using the Shapiro-Wilk test. Non-parametric tests (Mann–Whitney) according to the Gaussian distribution, were performed to detect significant independent variables The graphical results are shown as the median, first quartile, third quartile, and half dotted line. Sigma Plot® version 15 (Systat Software Inc., SanJose, CA 95131, USA, EE. UU, North First Street, Suite 360, Jandel Corporation, San Jose, CA, USA) was used to generate the analysis and graphs. Differences were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. General Characteristics the Cases and Controls

Table 1 describes the general characteristics of the three patients with IE and of the control subjects. A total of three women and three men were included with an overall median age of 66 (min 43-Max 83). The demographic characteristics and admission conditions are shown. Surgical interventions were performed in two cases of IE and one underwent TAVI. The three control cases were treated by surgery. In addition, the demographic and diagnostic characteristics of patients and controls are shown in Table 2.

Table 1 General characteristics of the three patients with IE and of the control subjects.

Table 1. Demographic characteristics of the three patients with IE that required aortic valve replacement surgery and controls subjects with aortic valve damage who required aortic valve replacement and did not develop endocarditis and that were included in the study. Abbreviations: NT-Pro-BNP= Natriuretic peptide. Abbreviations: NT-Pro-BNP= Natriuretic peptide.

	Endocarditis	Control Subjects
Age	70 (64-83)	65 (43-67)
Body Mass Index	22 (20-25)	29 (28-33)
Comorbidities		
Diabetes Mellitus	1 (33)	1 (33)
Systemic Arterial Hypertension	2 (66)	2 (66)
Dyslipidemia	1 (33)	1 (33)
Smoking	2 (66)	2 (66)
Laboratories		
Glucose	119 (85-131)	115(109-164)
Creatinine	0.81 (0.7-1.5)	1.4 (0.98-1.7)
Blood urea nitrogen	30 (22-41)	18 (15-23)
Uric acid	6.7 (3.8-7)	9.8 (5.3-10.1)
Natriuretic peptide NT-Pro-BNP	7339 (1971-22494)	200 (170-278)
Hemoglobin	11.8 (11.1-14.7)	14.4 (13.5-15.9)
Platelets	172 (152-200)	160 (91-230)
Leukocytes	8.4 (5.6-16.1)	8 (6.5-10)
Lymphocytes	1.1 (1-8.7)	2.5 (1.2-2.7)
Neutrophils	6.6 (4.5-13.9)	6.1 (3.1-7)
Erythrocyte sedimentation rate mm/	30 (27-43)	18 (16-32)
C-reactive protein mg/L	47.5 (40-200)	2.5 (0.7-6.9)
Total cholesterol	173 (138-325)	112 (108-186)
High Density Lipoprotein	53.4 (45.5-53.8)	44 (28.5-44.5)
Low Density Lipoprotein	98 (92-196)	122 (101-125)
Triglycerides	131 (130-211)	2123 (70-221)
Aorta diameters		
Aortic valve plane	29 (20-38)	26 (22-27)
Sinus of Valsalva	37 (20-40)	28 (21-58)
Sino tubular junction	38 (18-40)	27 (21-58)
Ascending aorta	37 (16-39)	30 (20-37)
Ejection fraction of the left ventricle	20 (20-45)	44 (30-65)

Table 2. the demographic and diagnostic characteristics of patients and controls. Abbreviations: DAoI= Double aortic injury, AVR= aortic valve replacement, AoI= Aortic insufficiency. Ao= aortic. LV= Left Ventricle. LVEF= Left ventricle ejection fraction, TAVI= Trans catheter aortic valve Implantation, MVR= mitral valve replacement, GLS= Global Strain, BAV= Atrioventricular block, PAP pulmonary arterial pressure, DM= Diabetes Mellitus, SAH= Systemic arterial Hypertension.

Case	Age	G	BMI	Diagnosis	Evolution	Death
1	55	F	20	2009 diagnosis of Arteritis de Takayasu plus bicuspid aortic valve SAH 1999 AVR by severe AoI (Medtronic Hall).	Endocarditis in 2016. univalve prosthetic valve with pannus and abscess with tissue destruction at level of interventricular septum with bacterial growth with *Staphylococcus aureus* and *epidermidis*. Surgery AVR and implantation of, dual-chamber pacemaker, by complete BAV, she had ischemic stroke and left hemiparesis with evolution with reduced heart failure LVEF 25% died in 2017 one year after surgery.	yes
2	67	M	30	2019 DAoI and AoI severe bicuspid aortic valve LV systolic and diastolic dysfunction LVEF 20%, smoking.	2020 native valve endocarditis with mobile vegetation of left non-coronary valve plus ascending aortic aneurysm, Surgery AVR Medtronic Hall with bacterial growth with *Streptococcus viridansj*. 2024 normal functioning prosthesis, LVEF 57%.	no
3	78	F	24	DAoI and stenosis Ao, tri-valve Aortic valve. 2019 March she received intervention with Transcatheter aortic valve implantation (TAVI) Portico 25 mm plus Boston Scientific DDD Pacemaker Tachycardia Bradycardia Syndrome Comorbidities: Smoking, systemic arterial hypertension, dyslipidemia.	2019 April she had TAVI endocarditis with vegetation in the aortic valve. Mitroaortic junction abscess plus aortitis aortic wall abscess. Valve crop *Enterobacter cloacae*, *Klebsiella Oxytocic* She was treated with aortic valve explanation surgery, ascending aorta resection. Bentall and Bo, MVR Edwards Peri mount. Mitroaortic command surgery. 2024 she is live LVEF 40%.	no
4	60	F	30	Ischemic heart disease, Tri valvular disease 2014 angioplasty in right coronary artery, 2017 aortic stenosis, AVR with mechanical prosthesis St Jude Masters HP 21, LVEF 52%. Comorbidities: Mellitus diabetes.	2024 asymptomatic LVEF 58%.	no
5	37	M	28	2018 Ascending aortic aneurysm aortic insufficiency, 4-cavity dilatation eccentric hypertrophy LV severe mild mitral insufficiency PAP 74 mmHg, Surgery AVR, DM, Systolic. arterial hypertension, dyslipidemia, hyperuricemia, positive smoking. LVEF 44%.	2019 Gout, asymptomatic cardiovascular LVEF 50%, systolic dysfunction GLS 14.5.	no
6	62	M	34	Ventricular dysfunction, severe aortic and mitral insufficiency, generalized hypokinesia LVEF 30%. 2014 Surgery due to Aortic Dissection Stanford A, DeBakey 1	2017 Asymptomatic, controlled high blood pressure stopped coming since that date.	no

plus Severe tricuspid regurgitation and mechanical AVR
St. Jude. SAH, Smoking.

The Figure 1 shown the echocardiographic findings cases with IE where in the case 1, is obsevate trans gastric short axis showing vegetation (yellow arrow) in relation to the sub valvular mitral apparatus. In the Case 2 left side, is showing the mitral valve endocarditis (yellow arrow), and right side is the same case, put is showing the severe mitral regurgitation. In the case 3, is showing where transesophageal short axis, in left side, and three chamber view, showing vegetation in the aortic valve (in right side, yellow arrow).
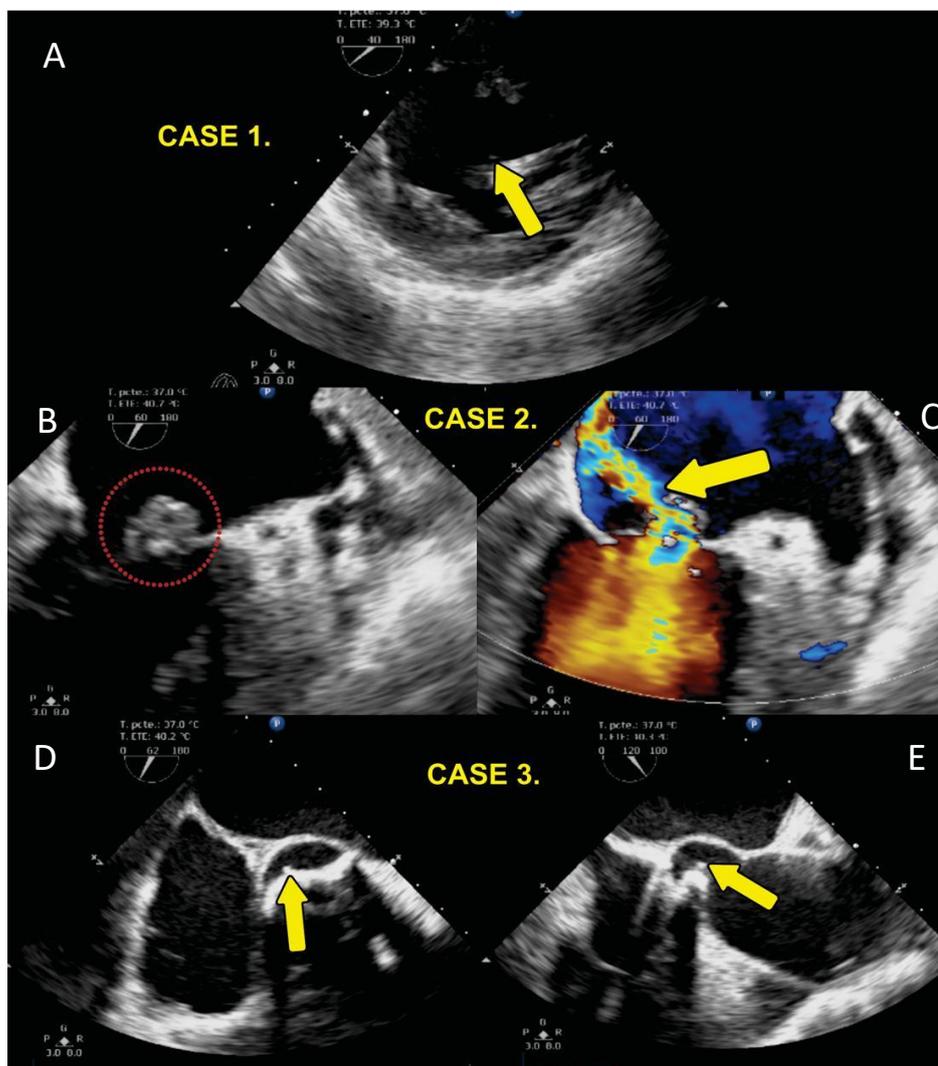
Figure 1. Transesophageal echocardiographic analysis of the patient series. Panel A Case 1. Trans gastric short axis showing vegetation (yellow arrow) in relation to the sub valvular mitral apparatus. Case 2 Showing mitral valve endocarditis (yellow arrow), panel B, and with severe mitral regurgitation, panel C. Case 3. Transesophageal short axis, panel D, and three chamber view, panel E, showing vegetation in the aortic valve (yellow arrow).

3.2. Oxidative Markers

The OS markers are shown in Figure 2. MDA was increased (p=0.01, panel A) and carbonyl groups showed a tendency to increase but without reaching a statistically significant difference (p=0.1, panel C) in the homogenized tissue of the aortic valve of the patients with IE in comparison with CS. The TAC and GSH (p=0.01, panel B and D respectively) were diminished in the patients with IE but the NO_3^-/NO_2^- ratio showed a decreasing trend. The thiol groups did not show significant changes (p=0.07, panel E and F respectively) in comparison to CS.
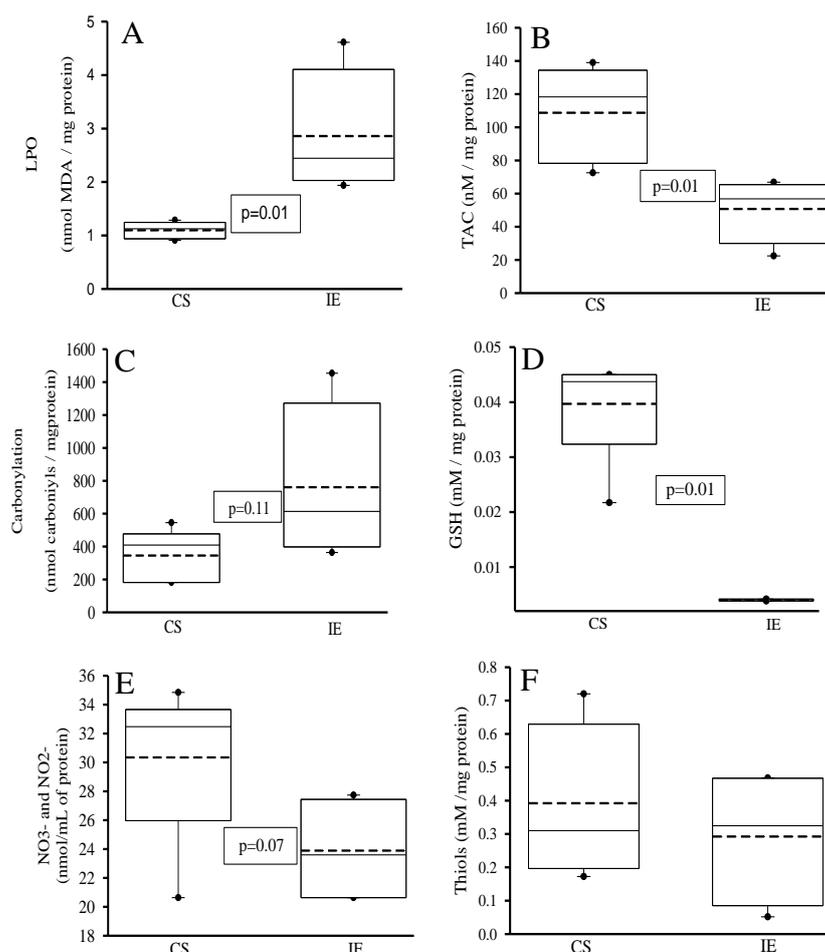
Figure 2. Oxidative stress markers such as MDA levels (A), TAC (B), Carbonylation (C), GSH (D), thiols groups (E) and NO_3^-/NO_2^- ratio (F) in homogenized of the valve cardiac of the CS and IE. The values are expressed such as median, first quartile, third quartile, and half dotted line. The dark circles that stand out from each bar are the outliers. Abbreviations: CS= Control subjects, IE= Infective endocarditis, MDA= malondialdehyde, TAC= Total antioxidant capacity, GSH= Glutathione, NO_3^-/NO_2^- nitrate and nitrite.

3.3. Activities of the Antioxidant Enzymes That Employ the Glutathione

Figure 3 shows that the GST activity in the homogenized tissue of the aortic valve was increased in IE in comparison with CS (p=0.01, panel A). However, the activities of the GPx, and TrxR only presented a tendency to increase (p=0.1, panel B and D respectively). The GR activity in IE versus CS did not show significant changes (panel C).
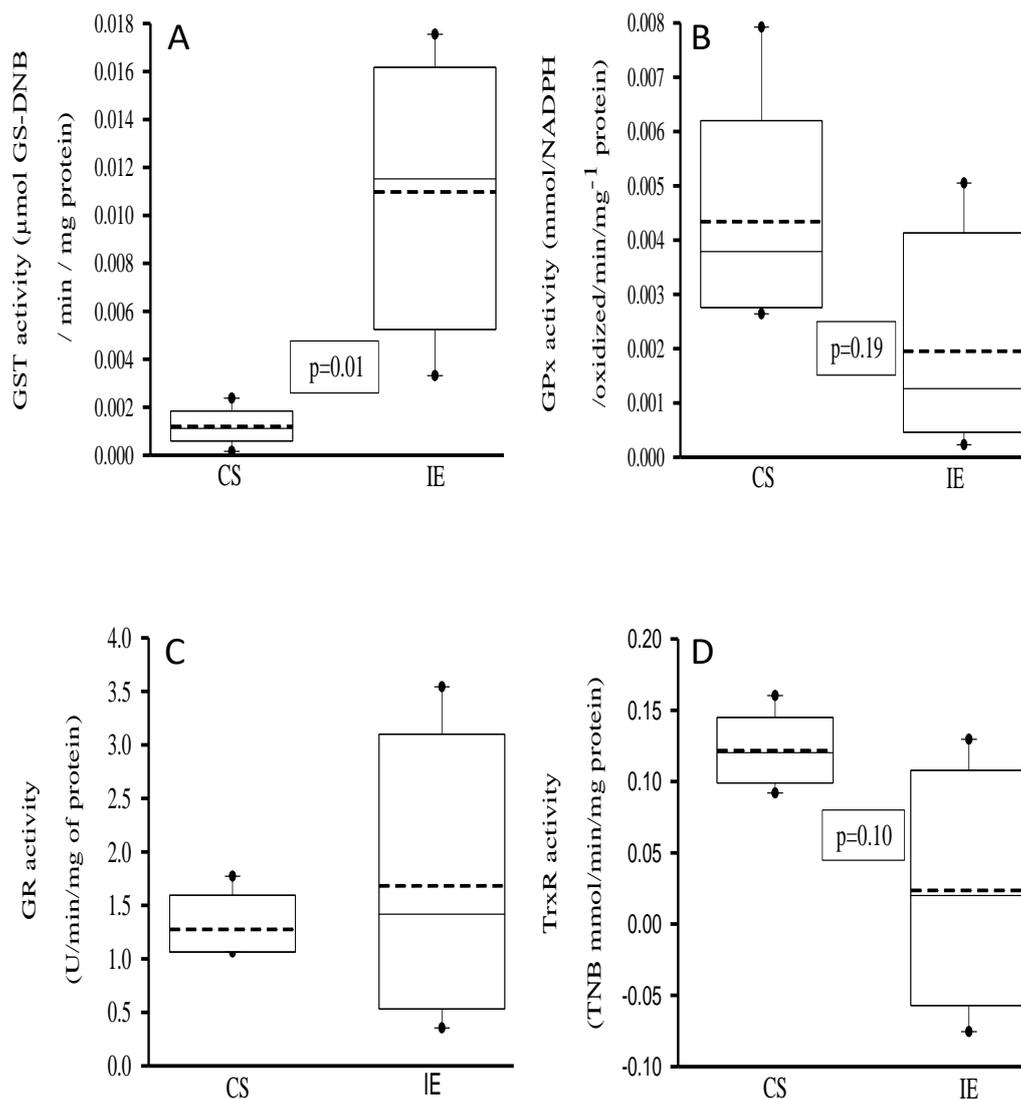
Figure 3. Determinations of some antioxidant enzymes that employ GSH such as GST (A), GPx (B), GR (C) and TrxR (D) in homogenized tissue of the aortic valve of the CS and IE. The values are expressed such as median, first quartile, third quartile, and half dotted line. The dark circles that stand out from each bar are the outliers. Abbreviations: CS= Control subjects, IE= Infective endocarditis.

3.4. Peroxidases and SOD Activities

The activities of the peroxidases (p=0.04) and SOD isoforms (p=0.04 and p=0.03) were increase in the homogenized tissue of the aortic valve of the IE patients in comparison with CS (Figure 4 A and B respectively).
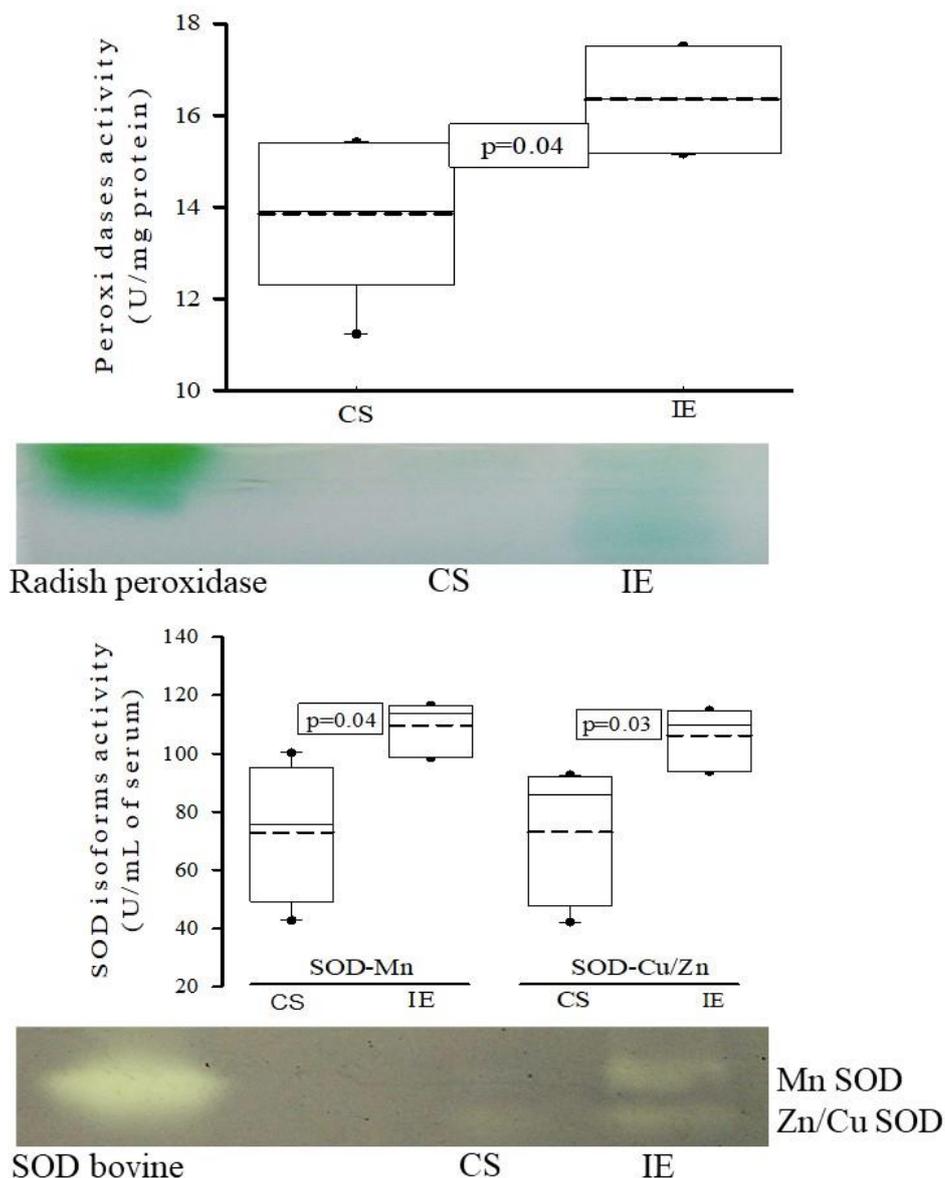
Figure 4. Activities of Peroxidases (A) and SOD isoforms (B) in homogenized tissue of the aortic valve. The values are expressed such as median, first quartile, third quartile, and half dotted line. The dark circles that stand out from each bar are the outliers. Abbreviations: CS= Control subjects, IE= Infective endocarditis, SOD= super oxide dismutase's.

4. Discussion

In this paper we evaluated the levels of OS markers and the enzymatic and non-enzymatic antioxidant systems in native or prosthetic valves from patients with IE that required aortic valve replacement and in controls with AVR or mitral valve without IE. We also describe the effect of different therapeutics and compare the results with those from subjects undergoing AVR without endocarditis.

The loss of the redox homeostasis in IE is characterized by overproduction of O_2^- and H_2O_2 that results from the bacterial infection and that increases the NADPH activity in cardiomyocytes of the aortic valve. This increase, in turn, favors the activity of the enzymes that are responsible for the detoxification of these ROS such as the SOD isoforms and the family of peroxidases [6,7]. Our results show that the activity of the SOD isoforms, which have Cupper/Zinc (Cu/Zn) and manganese (Mn) in the catalytic center (SOD Cu/Zn cytosolic and SOD Mn mitochondrial respectively), and which are necessary for the dismutation processes of O_2^- to H_2O_2 was increased in the homogenized tissue of

the aortic valve of the IE patients. This result was probably due to the excess O_2^- that may be provided by the over- activity of the NADPH associated to the bacterial infection. However, the activity of the SOD isoforms may favor the decrease in O_2^- that, in turn, induces a high concentration of H_2O_2. This elevated concentration is the substrate of other enzymes such the GPx and the peroxidase families. Our results showed that the GPx activity had a tendency to decrease but without reaching statistical changes. This could be due to the small number of cases, but also to the fact that bacterial infections can decrease the activity of endogenous GPx as a mechanism for survival to perpetuate the infectious process [16]. However, the activity of peroxidases was increased in IE patients. Bacterial infections can activate eosinophil peroxidase and the myeloperoxidases in neutrophils and monocytes [17]. These enzymes that contain a hemo-group in their catalytic centers that are effective tools against bacterial infections. They utilize H_2O_2 to catalyze the oxidation of halides to generate hypohalous acids which is a potent oxidant that capable of oxidizing membrane lipids, proteins, RNA and DNA of microorganisms. This leads to the killing of bacteria [18]. Our results show that the activities of peroxidases were increased in the homogenized tissue of cardiac valves in patients with IE. This suggests that the peroxidase activities are increased to counteract the bacterial infection.

In addition, the increase of the H_2O_2 can favor the formation of OH– that oxidizes polyunsaturated fatty acid of the cell forming oxidized lipid-peroxides such as MDA, a LPO marker. Our results showed that the level of MDA was increased in the homogenized tissue of the cardiac valve in patients with IE. This increase also favors the loss of the TAC. In this sense, the concentration of GSH is depleted in bacterial infections. The reduction of GSH in the host is needed since it is an antioxidant molecule that helps fighting the increase in ROS associated with the infection process. Furthermore, throughout the infection, the bacteria can use the host's GSH for survival, replication, virulence and other processes. In fact, *Streptococcus* consumes it for nutritional purposes [19]. Our results show that the GSH concentration was decrease in homogenized tissue of the cardiac valve of patients with IE, reinforcing what was mentioned above. Also, the loss of GSH favors the oxidant background in the patient with IE as well as the increase of LPO and the decrease of TAC. However, the loss of GSH can also be due in part by its demand. In this sense, the activity of GST, which is an enzyme that employs GSH, forming less toxic glutathione S-conjugates, including products of LPO in the process detoxification, was increased in the homogenized tissue of the aortic valve. A possible explanation may be that it constitutes a compensatory mechanism to eliminate LPO products such as 4-hydroxy-2-nonenal and bacterial LPS. In this sense, a study in pediatric population (9-17 age) infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus* that were causing chronic lung infection showed an increase of the GST [20]. Another study demonstrated that a mixture of GST-mu-class isoforms from the *F. hepatica* administered intraperitoneally 1 hour after a LPS injection, is capable of significantly suppressing the LPS-induced cytokine storm in a mouse model of septic shock [21]. Furthermore, the lack of significant changes in some the enzymes such as GR, TxrR, GPx and oxidative markers as thiols groups, NO_3^-/NO_2^- ratio and carbonyls groups could be due to the low number of patients with IE studied.

On the other hand, aortic stenosis is a disease that can occur in several etiologies. It is also generally found in the general population with risk factors such as age, obesity, dyslipidemia, among others. It is also widely associated with the presence of bivalve aortas and autoimmune processes. Regardless of the etiological clinical factor, once the subjects require surgery for an aortic valve replacement, the condition can be complicated by the presence of endocarditis. There are still gaps in the knowledge the mechanisms of this disease that interact with inflammation and one of them is OS. When choosing to conduct this preliminary study, we intended to determine the importance of evaluating oxidative deregulation and its importance for future clinical or cohort trials with the appropriate number of patients and sample sizes. Exploratory studies allow for the determination of whether the hypothesis deserves special attention to invest resources for long-term prospective studies when the frequency of the disease is not so high.

The cases selected in this study were carefully chosen for their clinical representativeness. In one patient, endocarditis was associated with the presence of autoimmunity. There are few reported cases

of this association [22,23]. The second case did not have an autoimmune condition and there are large studies of series of patients in similar conditions; however, little information on the participation of the OS process in the mechanism of damage in the infection is studied. The reports that are available [24] have been done in animal models, or at an experimental level in vitro but not in human tissues. In the third case, aortic stenosis was performed with interventional therapy, which is most commonly indicated in older subjects with adjacent comorbidities where the surgical risk is very high [25,26].

Regarding the specific characteristics of each patient, case number one, corresponded to a 55-year-old woman with an established diagnosis of Takayasu arteritis (TA), that developed endocarditis. The culture of the aortic valve reported the presence of *Staphylococcus aureus* and *epidermidis*, (supplementary material video). Therefore, in this case, IE was fully documented. Although there are few reported cases of large vessel vasculitis, the coexistence of vasculitis and IE may exist [27]. However, there are reports that TA can be confused with IE, especially when signs and symptoms resemble the inflammatory activity or when multiple aneurysms are found [28]. This case was unfortunate, and the patient died a year after the valve replacement, since during post-surgery she was complicated with a cerebral vascular disease. Although she was treated with a timely surgical intervention, comprehensive therapeutic management could improve results in patients with similar conditions. Cases of TA are rare, and it is difficult to have large series with the coexistence of IE. However, it would be important to evaluate the use of antioxidant as an adjuvant therapy in this complex condition where inflammation persists due to the disease and can be associated with an infectious and deregulation of the OS state. In this study, all patients had a high LPO and there are no previously reported similar studies. Therefore, this is a case to highlight.

The second case with IE, was a 67-year-old woman with a bicuspid valve. Large series of patients with this condition have demonstrated that a high percentage of IE may be present in them [29]. In this case, *Streptococcus viridians* was found as the main pathogen, which is also one of the most frequently found bacteria. This patient has a current survival of 4 years, and in her file, we did not find complete data on the therapeutic management she had received. Nevertheless, she was in an intensive therapy area where standard management is always implemented and there was an immediate surgical resolution, (supplementary material video).

The third case corresponded to a 78-year-old man who was treated for aortic stenosis with implantation of transcatheter aortic valve (TAVI). 48 hours after this procedure, he presented endocarditis and underwent surgery. This procedure is cutting- edge and IE occurs with low frequency in the early phase, before 30 days, (supplementary material video) [2]. The patient developed IE within the first 48 hours after implantation. In most published cohorts, *Enterococcus spp*. has been identified as the most common pathogen, followed by *Staphylococcus aureus* whose incidence is relatively low, ranging between 0.1 and 3% [30,31]. However, IE post-TAVI is an early complication, and it is associated with a poor prognosis, not only in comparison with native valve endocarditis but also in comparison to surgically replacement of valve endocarditis, regarding both valve dysfunction and patient mortality [2]. The clinical profile of most TAVI candidates includes elderly patients who are at a high surgical risk due to the presence of several comorbidities at the time of the intervention [32]. This also represents a risk that can enhance common IE pathogens and the presence of other opportunistic bacteria [33]. In the case of this patient, the complication due to IE was associated with the presence of *Enterobacter cloacae, Klebsiella Oxytocic*. However, it also highlights the moment at which a solution was tried to be implemented both therapeutically and by surgical intervention. Furthermore, the patient was taken to a surgery called Commando with a high risk of mortality. However, the patient survived for 5 years after the reintervention by surgery and the multidisciplinary management in which he received antioxidants such as vitamin C. Although there is not a follow-up study to evaluate the effect of the antioxidant therapy, the evolution and survival achieved after the critical period, confirms the findings from a large series carried out by our group where OS was controlled through standard management and adjuvant therapy with antioxidants.

On the other hand, the control subjects that were selected for comparison with subjects with IE were intervened for aortic valve replacement and they underwent the same procedures as the subjects

with IE. Although they are not healthy subjects and it is expected that they also present alterations in redox homeostasis, the difference between the groups lies in the presence of IE. It should be noted that subjects who were selected as controls and the patients with IE had different indications for aortic valve replacement and this could be a topic of discussion regarding the loss of redox homeostasis. However, the point of comparison lies in the presence and absence of the IE

5. Conclusions

The results from this series of three cases of IE in aortic valve suggest the loss of an intricate redox homeostasis system in patients with IE in the aortic valve may be favor the bacterial process, with an unfavorable result for the patient with the consequences leading to fatal outcomes. The surgical management of IE in expert hands improves these complex conditions; however, the success of the intervention must be accompanied by follow- up during the pre- and post-surgical periods aimed to reduce the metabolic conditions caused by the comorbidities in each patient. In addition to reducing the infectious and inflammatory state, the presence of OS state of deregulation requires consideration of adjuvant management with antioxidant therapy. The findings also justify future studies through clinical trials where adequate sample size is included to confirm and support this hypothesis.

Study Limitations. There are studies on IE in large series of patients, but none is focused on elucidating the participation of OS independently of the origin and comorbidities of the patient. Although the small number of cases may seem a limitation, it is not really so, since our objective was to carry out an exploratory study on this topic to determine its feasibility through prospective studies. Exploratory or preliminary studies are often recommended to previously evaluate which findings deserve attention without investing large resources.

Study Advantages. Even with a small number of cases, this is one of the few studies conducted on human valve tissue. The findings support that oxidative stress is involved in the mechanism of damage of IE as an alternative mechanism that deserves attention as a possible therapeutic treatment.

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