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Possible break-down of redox homeostasis in Beals-Hecht syndrome

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Beals-Hecht (BH) syndrome is a rare autosomal dominant disorder caused by a mutation of the *FBN-2* gene that codifies for fibrillin-2 (FBN-2). Its nosology includes congenital contractural arachnodactyly. The aim of this study was to evaluate the possible breakdown of redox homeostasis in the thoracic aortic aneurysm (TAA) from patients with BH. We determined OS markers such as malondialdehyde (MDA), total antioxidant capacity (TAC), carbonyl groups, glutathione (GSH), thiols the nitrate/nitrite ratio (NO_3^-/NO_2^-) and super oxide radical (O_2^-) by spectrophotometry in homogenized TAA from BH and the ascending fragment of the thoracic aorta (AFTA) from control subjects (CS). We also measured the activities of some of antioxidant enzymes such as GST, GPx, GR and TrxR. The super oxide dismutase (SOD) isoforms, catalase and peroxidase activities were evaluated by native polyacrylamide gels. The activities of the antioxidant enzymes GPx, TrxR, SOD isoforms, catalase and peroxidases were decreased in the TAA from patients with BH ($p \leq 0.04$) and the OS markers NO_3^-/NO_2^-, TAC and thiols were decreased($p \leq 0.04$). In addition, O_2^- was increased in patients with BH ($p = 0.02$). The results suggest a possible loss of redox homeostasis; this loss could be due to the decrease of some of the enzymatic antioxidant system´s enzymes and some antioxidants of the non-enzymatic system. In addition, the decrease in TrxR activity and the concentration of thiol groups could contribute to the alteration and instability of the FBN-2 protein.

Keywords Beals-Hecht syndrome, Oxidative stress, Redox homeostasis, Antioxidant enzymes, FBN-2

The Beals-Hecht (BH) syndrome is a rare pathology with an incidence of less than 1 case per 10,000 people, and it is manifested by congenital contractural arachnodactyly (CCA)[1]. It is an autosomal dominant disorder caused by a pathogenic variant of the fibrillin-2 (*FBN-2*) gene, that is located in chromosome 5q23.31. The fibrillin-2 (FBN-2) protein consists of five domains including a calcium binding site, an epidermal growth factor (cbEGF) binding site, an EGF-like structural domain, a glycine-rich region, a hybrid (hyb) domain, and an 8-cysteine repeat region[2].

Fibrillins are major constituents of the extracellular matrix (ECM) and provide connective tissue elasticity and mechanical force. They help in the communication between cells through integrin receptors, and modulate the concentration, presentation, activation and bioavailability of transforming growth factor (TGFβ) and bone morphogenetic protein (BMP) complexes[3]. Fibrillin's are extensively located in organs and tissues and serve as templates for the deposition of elastin during formation of elastic fibers (EF). They are essential for maintaining the integrity of tissues such as skin, blood vessels, lungs and the ocular ligament[4]. The nosology caused by the *FBN-2* mutation in patients with BH syndrome is characterized by flexion contractures of multiple joints including elbows, knees, hips, ankles, and with long spider-like fingers, CCA, camptodactyly, contracture of major joints, scoliosis, pectus deformities and crumpled ears. Occasionally, there may exist cardiovascular signs similar to those present in Marfan syndrome (MFS) and, rarely, aortic dissection[5]. The mutation in the *FBN-2* gene is the only one associated with CCA.

The nosology, the mutations of the *FNB-2* gene in BH and the consequences present in patients who suffer from this disease have been described in the literature. However, to date, no basic research has addressed whether these patients may have oxidative stress (OS), even if this condition is present in other diseases with disrupted

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fibrillin's such as MFS and Loeys-Dietz syndrome. Patients with these diseases show increased reactive oxygen species (ROS) and depletion in the antioxidant enzymatic system[6,7].

On the other hand, the redox homeostasis is of vital importance to maintain the balance between ROS and the enzymatic and non-enzymatic antioxidant systems in humans. A disturbance in any of these systems causes alterations of the redox homeostasis leading to OS. OS is characterized by an increase in ROS such as super oxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl groups (OH^-)[8]. In this sense, the disturbance of redox homeostasis could favor a chronic systemic oxidative background in patients with BH leading to a positive feedback circuit, and fibrillin instability, thus contributing to the anatomical alterations present in patients. Therefore, the objective of this preliminary study was to evaluate the possible break-down of redox homeostasis in the TAA from BH syndrome patients.

Materials and methods

Recruitment of the BH patients and control subjects

This was an observational and comparative study. Three BH patients and six control subjects (CS) that were attended in the aorta clinic of the National Institute of Cardiology Ignacio Chávez were included. The patients were classified with BH by the Ghent criteria. The research protocol was approved by the research and ethics committee of the National Institute of Cardiology Ignacio Chávez (protocol number 23-1366). Each BH and CS patient signed an informed consent form in accordance with the Helsinki Declaration, as amended by the Congress of Tokyo, Japan[9].

A segment of the ascending thoracic aorta aneurysm (TAA) was taken during the procedure of Bentall and De Bono from BH patients and stored at 4 °C[10]. This surgical technique consists of the replacement of the aortic valve root and ascending aorta with a Dacron tube, to which also both coronary arteries are anastomosed on the lateral faces and a valvular prosthesis[10]. Patients were considered candidates for surgery when they had ≥ 5 cm dilation and had been previously presented and discussed in a medical-surgical session or when they had attended the Institute for the first time with dilation and/or aortic dissection.

Inclusion criteria for BH patients included:

- Patients who met the Ghent criteria evaluated by a rheumatology expert and that required surgical intervention of the TAA. Also, patients with another type of cardiovascular surgery such as David type 5 techniques, which was agreed upon consensus in a medical-surgical session were included.
- Patients with and age of 18 years old or older.
- Patients belonging to any gender.

The exclusion criteria were:

- Patients that did not agree to sign the informed consent form.
- Patients who did not suspend oral nitrates, NSAIDs, statins, calcium antagonist.
- Patients with β-blocker intake for 7 days prior to obtaining the samples.
- Patients with neoplastic disease and/or associated infection.
- Subjects with a smoking history in the last 4 years.
- Patients with different phenotypic variants or phenotypes related to such as Shiprintzen–Goldberg, Ehler–Danlos, Alagille, Weill–Marchesani, Turner, Noonan, Marfan, MASS, and Loeys–Dietz (all its variants) syndromes.
- Patients with bicuspid aortic valve.
- Patients with autosomal dominant polycystic, kidney disease.

Control subjects (CS)

The CS were 18 years old or more and were previously evaluated by a cardiologist and a rheumatologist to verify that they did not have a connective tissue disease. They met the diagnostic criteria for severe aortic valve dysfunction, and there was a recommendation to consider surgical intervention because of dilation of the ascending aorta according to the image studies. Therefore, all patients underwent an echocardiogram, CT scan, or MRI to determine valvular and aortic damage before presenting the case to the cardiac team, who ultimately decided on the most appropriate surgical technique to use, and the type of valve to be implanted for valve replacement, which could be a biological or mechanical prosthesis. Therefore, the ascending fragment of the thoracic aorta (AFTA) used was obtained from patients that did not have aortic damage and did not undergo surgery for aortic stenosis, having had no connective tissue syndrome pathology diagnosed. In these patients, there was no suspicion of inflammatory disease or the presence of a degenerative disorders such as thyroid and autoimmune diseases, hypertension or diabetes mellitus. Also, the drugs that could interfere with the outcome of the study such as NSAIDs or lipid-lowering agents, were suspended in the perioperative period. Cases were dealt cautiously, to avoid including patients undertaking treatment with allopurinol, antioxidants, or probable inhibitors of ROS production. Warfarin, aspirin, clopidogrel, anticoagulant, antiplatelet medications, and other similar drugs were suspended.

The TAA segment from patients with BH and the AFTA in CS, that were obtained during surgery were sectioned into two portions, placed in tubes and transported under Liquid nitrogen and frozen at -70 °C until study. The studies requested during hospitalization for both BH patients and CS included the determination of triglycerides (TG), HDL, LDL, total cholesterol (CT), creatinine, and glucose. All assays on serum biochemical variables were made in duplicate.

Thoracis aorta homogenization

The segment from the TAA and the AFTA of the BH and CS respectively, was homogenized under liquid nitrogen after adding KH_2PO_4 (2 mL) pH 7.3 in presence of 20 µL antiprotease inhibitors (1 mM PMSF, 2 μM pepstatin A, 2 μM leupeptin, and 0.1% aprotinin), and the preparation was kept in ice. The Bradford method was utilized to determine the protein concentration in the homogenates[11].

Oxidative stress markers

Determination of malondialdehyde

Malondialdehyde (MDA) was read spectrophotometrically at 532 nm. A quantity of 100 µg of homogenized tissue from the TAA of BH patients and the AFTA in CS were used. Methanol with BHT at 4% (100 µL) plus KH_2PO_4 buffer pH 7.4 (1 mL) was added to the sample and it was then incubated at 37 °C for 30 min. Then, 2-thiobarbituric acid at 0.8 M (1.5 mL) was added. After that, the sample was incubated at 90 °C for 1 h. Then, KCL at 5% (1mL) plus n-butanol (4 mL) were added and shaken for 30 s, and the sample was centrifuged at 4000 rpm (2 min). The butanol phase was extracted, and the absorbance was measured[6].

Evaluation of the total antioxidant capacity

The total antioxidant capacity (TAC) was detected spectrophotometrically at 593 nm. 100 µg of homogenized tissue from the TAA and the AFTA of the BH and CS respectively, were incubated in the 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-2pyridyl-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[6].

Carbonylation

To evaluate the amount of carbonyl groups, 100 µg of homogenized tissue from the TAA and the AFTA of the BH and CS respectively were used. The sample was 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 samples were centrifuged at 15,000× *g* for 5 min. The bottom was recuperated, and it was washed two time, adding $C_2H_5OH/C_4H_8O_2$ (1 mL). Then, the samples were 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) was added and the mixture was incubated at 37 °C for 30 min. The absorbance was read spectrophotometrically at 370 nm[6].

GSH and thiols

For the GSH determination, 100 µg of homogenized tissue were used and its presence was detected spectrophotometrically at 412 nm, according to the Ellman's method. Thiol groups were read spectrophotometrically at 415 nm. 50 µg of homogenized tissue form the cardiac valve of BH patients and CS were used according to Erel and Neselioglu's method[7].

NO_3^-/NO_2^- ratio determination

To evaluate the NO_3^-/NO_2^- ratio, 100 µg of homogenized tissue from the TAA and the AFTA of the BH and CS respectively, previously deproteinized with 0.5 N, NaOH and 10%, $ZnSO_4$ (100 µL) were used. Then, 10 µL of the cytochrome c reductase (NADPH) (Sigma Aldrich Cat# 24479) were added and the samples were incubated for 30 min at 37 °C. Sulfanilamide 1% and N-naphthyl-ethyldiamine 0.1% (200 µL respectively) were added and the total volume was adjusted with distilled water to 1 mL. The absorbance was measured at 540[7].

Detection of the super oxide anion

The O_2^- was determined in the homogenized tissue from the TAA and the AFTA of the BH and CS respectively. Briefly, 50 µg of the homogenized tissue were added to glycine buffer 2 mL (50 mM,) at pH 10.2 plus epinephrine 50 µL (60 mM) and incubated at 30 °C for 8 min. The indirect detection of the O2– by irreversible oxidation of epinephrine to adrenochrome occurs at 1 to 5 mmol/min–1 and was monitored spactrophotometrically at 480 nm with an extinction coefficient of 4.0 mM^{-1} cm^{-1}.in the homogenated of the TAA and the AFTA of the patients with BH and CS respectively.

Determinations of antioxidant enzymes that employ GSH as a substrate

To evaluate the activities of GST, GPx, GR and TrxR, 100 µg of homogenized tissue from the TAA and the AFTA of the BH and CS respectively, were used and treated as previously described[10]. 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. The activity TrxR was determined after incubation and monitoring 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}[18].

Determinations of super oxide dismutase isoforms, catalase and peroxidases activities

The super oxide dismutase (SOD) isoform, catalase and peroxidase activities were determined through non-denaturing gel electrophoresis[10]. 100 µg of homogenized tissue from the TAA and the AFTA of the BH and CS respectively, were applied directly in non-denaturing 10% polyacrylamide gels. The electrophoresis was carried out at 120 volts for 4 h. For SOD isoform's activity, 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. For catalase activity the gel was incubated with a mixture of 1% $K_3Fe(CN)_6$ and 1% of $FeCl_3$ $6H_2O$ for 10 min in the dark and then washed with distilled water to stop the reaction[12]. Purified SOD from bovine erythrocytes with a specific activity of 112 U/mg of protein (Sigma-Aldrich, St. Louis, MO, USA) and purified CAT from a bovine liver having a specific activity of 60 U/mg (Sigma-Aldrich) were used as positive control to calculate 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 of the SOD isoforms, catalase and peroxidase gels were analyzed using densitometry with a Kodak Image® 3.5 system. The samples were placed in a separate lane of the gel and run in parallel with the biological samples. The intensity of the signal from the control lanes was used as a reference to measure the enzymatic activity in the tissue samples. Therefore, the results are expressed as U of activity per mg of protein. The gels were analyzed by densitometry with image Sigma Scan Pro 5.1 software (Systat Software, Inc., San Jose, CA, USA).

Statistical analysis

Continuous variables are expressed as median with minimum and maximum values. Categorical variables, such as frequencies and percentages, are reported. The results were normalized with a natural logarithm and the normality distribution was evaluated using the Shapiro-Wilk test. The Mann–Whitney test used according to the Gaussian distribution, was performed to detect significant independent variables The graphical results are shown as the median, first quartile, third quartile, and half dotted line. The Sigma Plot program (Systat Software Inc., SanJose, CA 95131, USA, EE. 191 UU, North First Street, Suite 360, Jandel Corporation, San Jose, CA, USA) was used for statistical analysis. The GraphPad-Prism 8 Software. Inc. (San Diego, CA, USA), 1995–2023, was used to generate the graphs. Differences were considered statistically significant when $p \leq 0.05$.

Results

Table 1 describes the positive characteristics of the Ghent criteria in the three patients with the BH syndrome. The three cases included were men with an overall median age of 38 (min 30 – max 66).

Table 2 describes the clinical and symptomatic characteristics of the three patients with BH and the six control subjects.

Table 3 shows the demographic characteristics, comorbidities, serum biochemistry and general characteristics of the aorta in the three patients with BH and the six CS patients

OS markers of the non-enzymatic system are shown in (Fig. 1). In this study we determined NO^{3-}/NO^{2-} ratio, TAC, carbonylation, GSH and thiol groups which were decreased in patients with BH syndrome in comparison with CS ($p \leq 0.001$ panel a and, $p = 0.04$ panel e, $p = 0.01$ panel f, respectively). The carbonyl groups and LPO-index were not significantly modified between groups.

Figure 2 shows that the GR, GPx and TxrR activities in the homogenized tissue from the TAA of HB patients was decreased in comparison with CS ($p = 0.04$ $p = 0.01$ and $p < 0.001$, panel a, b and d respectively). However, the activity of the GST did not change in BH in comparison with CS (panel c).

The activity of the SOD-1 and −2 ware decrease ($p = 0.02$ and $p = 0.01$ Fig. 3A, b, respectively) in BH in comparison with CS (Fig. 3b).

The peroxidases and catalase activities showed a decrease in BH in comparison with CS (Fig. 4a, b, $p = 0.01$).

The Fig. 5 present the indirect detection of the O_2^- by irreversible oxidation of epinephrine to adrenochrome, showed an increase in the patients with BH in comparison to CS, $^*p = 0.02$.

Discussion

The alteration of the redox homeostasis in the TAA from patients with MFS and in some of its variants such as the Loeys-Dietz syndrome has been previously demonstrated[12–14]. However, there is no information on the alteration of redox homeostasis linked to an increase in the oxidant background, in patients with the BH syndrome which might be associated with the *FBN-2* gene mutation. Therefore, the aim of this study was to demonstrate whether there is an alteration of the redox homeostasis in patients with BH syndrome.

The clinical identification of MFS is accomplished through the Ghent criteria or genetic mapping; however, there are only clinical data that guide the physician to suspect the diagnosis of BH syndrome. In this series of cases, we were able to differentiate patients with MFS from those with BH syndrome through the scoring system proposed by Meerschaut et al.[15]. All three cases met the Ghent nosology and score for the diagnosis of BH and, although the gold standard test is genetic mapping, only the third case was confirmed by genetic testing of a positive *FBN-2* mutation. The scoring method used allows for the classification and suspicion of the diagnosis with a sensitivity of 75% and specificity of 60%, when a genetic determination is not available[15].

The overlap of several clinical conditions in MFS and BH syndromes can be explained because both conditions present mutations in two homologous genes, the *FBN-1* and *FBN-2*, with similar functions. Nevertheless, the altered gene in MFS encodes for FBN-1 and is located chromosome *15q15-21.3* while the modified gene in patients with BH syndrome is in the *5q23-31* chromosome and encodes for FBN-2[15,16]. Both mutations are related to skeletal, cardiovascular and aortic damage, and reports of cardiac abnormalities in BH syndrome have appeared for more than 4 decades. Mutations of both fibrillin's have been found in a single patient and reported in the literature. This situation can synergistically worsen cardiovascular manifestations and these patients also present other comorbidities16.

	Case 1	Case 2	Case 3
Age at the time of diagnosis	29	53	25
Age (current post surgery and intervention)	30	66	36
Gender	Man	Man	Man
Ghent criteria > 2 is required for classification			
MFS family history	No	No	No
Aortic root dilation (mm)	41	31	19
Sinus of Valsalva dilation (mm)	91	52	27
Sino tubular junction dilation (mm)	90	38	24.7
Ascending aorta dilation (mm)	46	37	28
Abdominal aorta	Without dilation	33	36
Aortic dilatation	Yes	Yes	yes
Lens dislocation	No	No	No
Systemic score			
Facials	1	1	1
Steinberg and walker murdock sign	0	0	3
Pectus carinatum	0	2	0
Pectus excavatum or asymmetry of the thorax	1	0	0
Hollow foot	0	0	0
Normal or flat foot	1	0	0
Pneumothorax	0	0	0
Dural ectasia	0	2	0
Acetabular protrusion	0	0	0
US/UL Reduction or stroke/height ratio	1	0	0
Mild scoliosis	0	0	0
Thoracolumbar scoliosis or kyphosis	1	1	0
Reduction of elbow extension	0	0	0
Skin with stretch marks	1	0	0
Myopia > 3 diopters	0	0	0
Mitral valve prolapses	0	0	0
Total systemic score	5	6	4
Positive systemic score (> 7)	No	No	No
Positive genetic test for *FBN-1*	No	No	No
	1 = 2, aortic dilatation	2 = 2, aortic dilatation	2 = aortic dilatation and in his mother
Clinical suspicion of the BH syndrome by score system			
Appearance of ear helix	Wrinkled (3)	Wrinkled (3)	Elf (3)
Highly arched palate	Yes (1)	Yes (1)	Yes (1)
Retrognathia	Yes (1)	Yes (1)	Yes (1)
Joint contractures	Hands and feet (3)	Hands and feet (3)	Hands and feet (3)
Arachnodactyly	No	no	Yes (3)
Dolichostenomelia	Yes (2)	Yes (2)	No
Pectus deformity	Yes (2)	No	No
Kyphoscoliosis	Yes (1)	Yes (1)	No
Mutation *FBN-2*	No done	No done	Positive
Total score system	13	11	11 + mutation

Table 1. Shows positive characteristics of the Ghent criteria in the patients with BH syndrome.

In this sense, FBN-2 participates in the formation of the embryonic valve and is pointed out as a molecular factor involved in bicuspid aortic valve complexity[15]. The patients with BH in this series of cases also had severe dilatation of the roots of both great arteries with aortic insufficiency[16].

Likewise, patients with only CCA, as in BH syndrome seem to have a favorable prognosis. Nevertheless, an association of the presence of aortic dilation and aortic dissection with some polymorphisms in the *FBN-2 gene* have been identified in some cases[17]. Timely clinical detection and imaging studies make possible the early detection of the possible aortic disease, and *FBN-2* genetic testing could be useful to identify cases[17].

Patients with BH have aortic root dilatation and the vast majority lack evidence of congenital heart disease. In addition, the *FBN-2* gene spans more than 28 kb and 65 exons and is expressed early in development being constrained to bone and cartilage matrices. This gene is essential for the EF formation in adult tissues. Ten novel mutations have been identified in the *FBN-2* protein in patients with BH, and 75% of them happen in

BH case	Sex	Age	Description
1	Man	30	House painter by occupation. At the age of 28 he began to have dyspnea and chest tightness. He was evaluated by a cardiologist for recurrent oppressive chest pain, and was sent for evaluation to our center. On admission, at 29 years of age, he reported chest tightness. The echocardiographic study showed that he had aortic insufficiency, dilated left cavities, and decreased systolic function. The computed tomography also found severe destroxone convex scoliosis, aneurysm in the aortic root and ascending aorta. At the age of 30, he underwent Bentall and De Bono surgery. He was evaluated for suspected MFS; the only positive Ghent criterion was aortic dilation. He is currently alive, with 9 years of evolution. LVEF of 58%.
2	Man	66	A farmer, who began to have dyspnea when he was 53 years old and sought medical attention 6 years later. He was found to have a marfanoid habitus and aortic insufficiency attributed to aortic dilatation. A CT scan showed dilatation of the aortic root. The sinuses of Valsalva of 52 mm and an abdominal aortic aneurysm of 33 mm. He only met one of the Ghent criteria (aortic dilatation) and only met 6 on the systemic score. He had no other antecedents. His clinical characteristics showed a deep high-arched epicanthus palate, wrinkled ears, severe scoliosis and pectus carinatum. He underwent surgery using the Bentall and De bono technique. He is currently 66 years old and is stable. LVEF 62%.
3	Man	36	A financial stockbroker who practices at Parco. At the age of 25, on a trip to Israel, he presented acute abdominal pain that radiated to the pelvic cavity. A CT scan showed an abdominal aneurysm, that was treated with an endoprosthesis. He was evaluated for suspected MFS. The only relevant history was the death of his mother due to aortic rupture, but she died without a diagnosis. The patient only met one Ghent criterion (aortic dilatation) and was positive for mutation in the *FBN-2 gene*. The patient's clinical data included epicanthus, elf ears, CCA in the hands and feet, and a diagnosis of BH syndrome was concluded. Six years later, he presented a right femoral iliac dissection that was treated with an endoprosthesis. It was complicated by compartment syndrome treated with fasciotomy and finally had to be resolved with supracondylar amputation. Currently, he is stable. LVEF 64% without aortic dilatation in the thorax.

CS	Sex	Age	Description
1	Man	72	Foreign farmer with no significant degenerative or cardiovascular history in 2014. At the age of 72 years, he began to have intense dominant pain, with straining and tenesmus. An ultrasound was performed in a private hospital, which showed an abdominal aneurysm. He was referred to our Institute and a CT scan was performed, which showed a ruptured infrarenal abdominal aneurysm with hemoperitoneum (Crawford-IV). Emergency surgery was performed. An 18 × 30 mm aortic graft was placed. The patient is alive and has a postoperative evolution of 10 years and two months.
2	Man	52	Foreign farmer with a history of arterial hypertension of 10 years of evolution and dyslipidemia, alcoholism and positive smoking. His condition began at the age of 52. In 2015, after his work- day, he had intense precordial pain 7/10. In his place of origin, they performed a computed tomography that showed aortic dilatation and dissection. He was sent to our Institute and the MRI showed aortic dissection Stanford IIIB De DeBakey. Aortic dilatation of the descending thoracic abdominal aorta of 75 mm. The left ventricle showed concentric hypertrophy. Surgery was performed with aortic-thoracic abdominal replacement and revascularization of abdominal trunks. He died 24 h later with refractory metabolic acidosis and acute kidney injury III.
3	Woman	48	Woman with housework occupation. In 2011 at the age of 40 she had dyspnea and was studied by a cardiologist who diagnosed her with significant aortic insufficiency and proposed aortic valve replacement. However, the patient postponed the surgery. In 2016 the dyspnea had increased, she was found to have severe aortic insufficiency and a computed tomography found a bivalve aorta and dilation of the ascending aorta. Bentall and Bono surgery was performed. Current surgical survival is of 8 years and 4 months.
4	Woman	30	Woman with housework occupation, with body mass index of 60, ischemic heart disease, tri-valvular disease. In 2014, angioplasty in right coronary artery was performed and in 2017 she presented aortic stenosis, AVR with mechanical prosthesis St Jude Masters HP 21, LVEF 52%. However, in 2024 she presented asymptomatic LVEF 58%. Also presented diabetes mellitus as a comorbidity.
5	Man	28	Man with a body mass index of 37. In 2018, he was sent to our Institute presenting an ascending aortic aneurysm with aortic insufficiency, 4-cavity dilatation eccentric hypertrophy LV, severe mild mitral insufficiency, PAP 74 mmHg, surgery AVR, LVEF 44%. In 2019, gout was present, asymptomatic cardiovascular LVEF 50%, systolic dysfunction GLS 14.5. He also presented diabetes mellitus, arterial hypertension, dyslipidemia and hyperuricemia such as comorbidities and a positive smoking.
6	Man	34	Man with body mass index of 62 that presented ventricular dysfunction, severe aortic and mitral insufficiency, generalized hypokinesia, LVEF 30%. In 2014, he underwent surgery due to aortic dissection, Stanford A, DeBakey 1 plus severe tricuspid regurgitation and mechanical AVR St. Jude. He also has some comorbidities such as hypertension, and a positive smoking habit.

Table 2. Description of the clinical and symptomatic characteristics of the patients with BH and CS. *MFS* Marfan syndrome, *LVEF* left ventricular ejection fraction, *CT* computed tomography, *CCA* contrast clearance analysis, *AVR* aortic valve replacement, *LV* left ventricular, *GLS* global longitudinal strain.

the consensus region. Interestingly, none of these identified *FBN-2* mutations alter amino acids in the calcium-binding sequence or in the EGF-like domains. The pathogenic variant causes a decrease in the amount of the FBN-2 protein available for the formation of microfibrils; hence, it reduces the EF, resulting in the symptoms exhibited in patients such as reduced strength and flexibility to the connective tissue that sustains the body's joints and organs[18]. These findings have led to the search of a better understanding of the molecular basis of CCA and the mechanisms of damage that interact with the inflammatory status and oxidative dysregulation[13].

On the other hand, EF assembly is a multistep process in which tropo-elastin (TE) monomers are secreted from elastogenic cells and then self-assemble/coacervate to form microaggregates that interact with fibulin-4 (Fbln) and − 5. This is a fundamental initial step for the generation of mature EF. In this process, the amino acids in TE could be susceptible to oxidative modifications and thus affect EF. Therefore, carbonyl formation by ROS on side chains of specific amino acids such as Lys, Arg, Pro, and Thr may occur, resulting in an alteration of protein structure and function. For example, oxidative modifications by O_2^- to collagen cause a change in the elasticity of the skin, as well as stiffer and more brittle cartilage[19]. The above supports the theory that each protein interaction during assembly could actively participate in the structural alteration observed in patients with aortic dilatation and dissection.

Our results only show a tendency to a decrease in the amount of carbonyl groups in the patients with BH. A possible explanation of this could be the small sample of patients studied. However, the amino acids in TE are susceptible to oxidative modifications and this may favor carbonylation which is associated with a decrease in TAC. In addition, FBN-2 contains two hyb domains composed of a TB/8-Cys-rich glycoprotein region with disulphide bridges between the Cys which are necessary for the temporal and hierarchical assembly of microfibrils that are indispensables to sequester TGFβ[20]. The formation of intermolecular disulphide bonds is not convenient between a conserved extra Cys in the hyb1 domain as an early part of the microfibril assembly process in cbEGF domains. This may result in reduced calcium affinity, and destabilization of the linkage of the cbEGF domain[21–23].

	BH ($n=3$, median and min -max range)	CS ($n=6$, median and min -max range)
Age (years)	38 (30–66)	41 (28–72)
BMI (weight/height^2)	25 (19–27)	31 (30–34)
Comorbidities (%)		
DM	0 (0)	2 (33.3)
SAH	0 (0)	4 (66.6)
Dyslipidemia	0 (0)	2 (33.3)
Smoking	0 (0)	3 (50.0)
Alcoholism	0 (0)	1 (33.3)
Serum biochemicals		
Leukocytes (10^3/μL)	6 (4.7–6.8)	6.8 (0.9–9.0)
Lymphocytes (10^3/μL)	1.9 (1.9–2)	1.9 (1.6–2.2)
Neutrophils (10^3/μL)	3.3 (2.2–4)	5.9 (4.4–7.1)
Platelets (10^3/μL)	194 (191–316)	268 (158–307)
Hemoglobin (g/dL)	15.9 (15.7–16.1)*	11.4 (10.1–13.2)
Glucose (mg/dL)	91 (90–100)*	107 (80–264)
Creatinine (mg/dL)	0.80 (0.75–0.88)*	0.83 (0.70–1.5)
BUN (mg/dL)	15.6 (11 – 4–20.6)	17.3 (10.3–21.7)
Uric acid (mg/dL)	6.4 (5.8–6.6)	8.1 (6.2–8.4)
Albumin (mg/dL)	4.3 (4–4.7)*	3.6 (2.4–3.6)
CT (mg/dL)	181 (167–188)	124 (98–146)
LDL (mg/dL)	120 (114–120)	81 (54–95)
HDL (mg/dL)	41 (34–49)	29 (16–34)
TG (mg/dL)	104 (92–218)	92 (80–127)
CRP (mg/dL)	0.58 (0.30–0.75)*	81 (6.5–143)
General characteristics of the aorta (mm)		
Aortic ring	31 (21–41)	25 (24–30)
Valsalva sinuses	52 (28–91)*	46 (30–48)
Sino tubular junction	38 (27–90)*	38 (28–56)
Ascending aorta	37 (29–46)	30 (30–82)
Descending aorta	33 (21–33)*	50 (26–54)
Mitral Regurgitation (%)	50 (30–60)	54 (52–55)

Table 3. Demographic characteristics of patients with BH and CS. *CT* cholesterol, *DM* diabetes mellitus, *SAH* systolic arterial hypertension, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *TG* triglycerides.

The TrxR/Trx system is fundamental for the equilibrium of redox homeostasis and disulphide bond regeneration. TrxR reduces thioredoxin (Trx) through GSH oxidation in the presence of $NADPH^+$. This results in reduction of disulfide bridges (thiol groups) between Cys. It contributes to reduce the protein dysfunction, inactivate cellular receptors, and the loss of enzyme activity[24]. Three TrxR isoforms (nuclear, mitochondrial and cytosolic) have been described in mammals. High and low expressions of Trx in the abdominal aorta aneurysms in the luminal and abluminal faces of arteries have been reported[25]. An efficient TrxR/Trx system contributes to decreases endothelial dysfunction and is associated with an anti-thrombotic and proinflammatory state. However, the alteration of the TrxR/Trx system contributes to OS resulting in disturbances in redox homeostasis[7]. Our results show a decrease in both the TrxR activity and in the concentration of thiol groups in the homogenized tissue from the TAA of arteries from BH patients. These results suggest that the TrxR/Trx system is highly compromised in this syndrome and might contribute to the decreased maturation of EF[26]. These changes could also be associated to the lack of disulphide bonds formed between TB/8-Cys-rich glycoprotein region of the FBN-2[27]. In addition, the low TrxR activity could also contribute to OS which is favored, in part, by the increase in the ROS including H_2O_2.

Moreover, one of the enzymes that uses H_2O_2 as a substrate in the presence of GSH is GPx. Our results showed a decrease in the activity of this enzyme. This suggests that this decrease could be due to the high concentration of intracellular H_2O_2 (enzymatic activity inhibition by substrate)[28]. GPx regulates the H_2O_2 concentration, having a modulating effect on the TGF-β1 signaling pathways[28], GPx is also capable of acting as a peroxinitrite ($ONOO^-$) reductase. A decrease in its activity could cause the accumulation of RNS in the aortic smooth muscle cells of patients as has been described in MFS[29,30]. Furthermore, $ONOO^-$ affects the enzymatic activities by introducing tyrosine residues or oxidizing thiol groups thereby leading to 3-nitrotyrosine formation or formation of oxidized thiol groups in proteins. $ONOO^-$ is the result of the oxidation of the nitric oxide (NO) by O_2^-[31]. Our results show a decrease in the NO_3^-/NO_2^- ratio and this reduction could be due in part to the rapid oxidation of NO to $ONOO^-$.

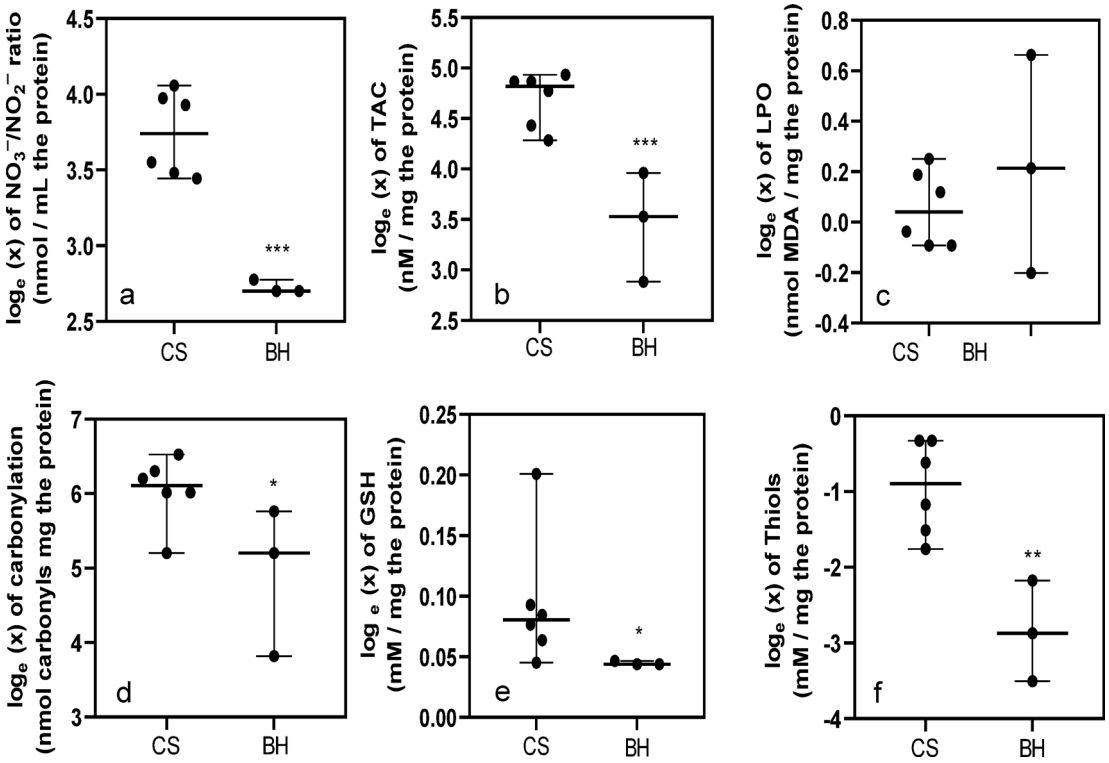

Fig. 1. Oxidative markers of the non-enzymatic system in the TAA and the AFTA of the patients with BH and CS respectively. *$p = 0.04$, **$p = 0.01$ and ***$p = 0.001$.

In addition, TE is susceptible to oxidative modifications by ONOO−, as is shown in an immunoblot analysis where TE is oxidized by the ONOO − and hypochlorous acid by activated monocytes and macrophages. In this sense, an in vitro study in an EF assembly model showed that oxidated modified TE failed to form EF through the cross-linking with other proteins required for EF assembly, including Fbln-4, -5, and FBN-2[32]. This suggests that the ROS/RNS ratio can modify the TE and that these modifications may affect EF assembly. Therefore, OS influences structural and functional changes in EF under pathological conditions[32]. However, more studies are necessary for the confirmation of this hypothesis.

On the other hand, the tripeptide GSH inactivates ROS by itself and it is a major thiol-disulfide redox buffer and antioxidant molecule that protects from overproduction of ROS under pathological conditions[33]. In addition, TxrR, GPx and GST require GSH as cofactor for ROS detoxification. This detoxification reaction converts reduced GSH to oxidized GSSG[34]. Our results show that GSH is decrease in the TAA from BH patients, and they suggest that the increased oxidative background in patients with BH may deplete this antioxidant molecule, contributing to the loss of the activity of TrxR and GPx favoring the oxidative background.

The reduction of GSSG to GSH is catalyzed by GR. This enzyme acts as a control mechanism for the regeneration and re-establishment of the GSH concentration[35,36]. Our results show that the activity of GR tends to decrease in patients with BH syndrome. The non- significant tendency could be due to the small sample size. These results suggest that the chronic state of OS may favor the GSH oxidation through the loss of GR activity which may result in a decrease of the GSH regeneration[37–39].

Regarding the isoforms of SOD, catalase and peroxidases, which are enzymes that participate in redox homeostasis, there are alterations in MFS syndrome that contribute to OS[7,8]. The results in this study show the presence of decrease activities in the native polyacrylamide gels of SOD-1 (the manganese isoform which predominates in the mitochondria and is very important in the muscular function to detoxicate ROS) and SOD-2, which is found in the cytoplasm (Cu-Zn isoform). Both enzymes are key in the detoxification process of O_2^-. The decrease in the activity of both isoforms in the patients with BH suggests that O_2^- contributes in an important manner to the oxidative background. It contributes to the generation of other ROS that have a longer half-life and are more aggressive in the oxidation process such as H_2O_2, OH^- and $ONOO^-$. In addition, the activities of the peroxidases and catalase (both responsible for detoxifying H_2O_2), showed a significant decrease. These results suggest that the patients with BH course with an increased oxidative background in the TAA[7,8,12–14], and this contributes in part to instability of the FBN-2 protein and EF.

In this sense, the NADPH oxidase family in MFS is increased and it participates in the formation and progression of TAA, through the induction of inflammation, MMP activity, VSMC apoptosis and changes in ECM properties[40]. This oxidase family is one of the main sources of O_2^- production. Our results in this study showed an increase in this ROS specie in the patients with BH compared to CS. This suggests an oxidative background that is associated with the alteration of the enzymatic and non-enzymatic antioxidant system and could lead to the modification of redox homeostasis thus contributing to oxidative modifications in TE. In this

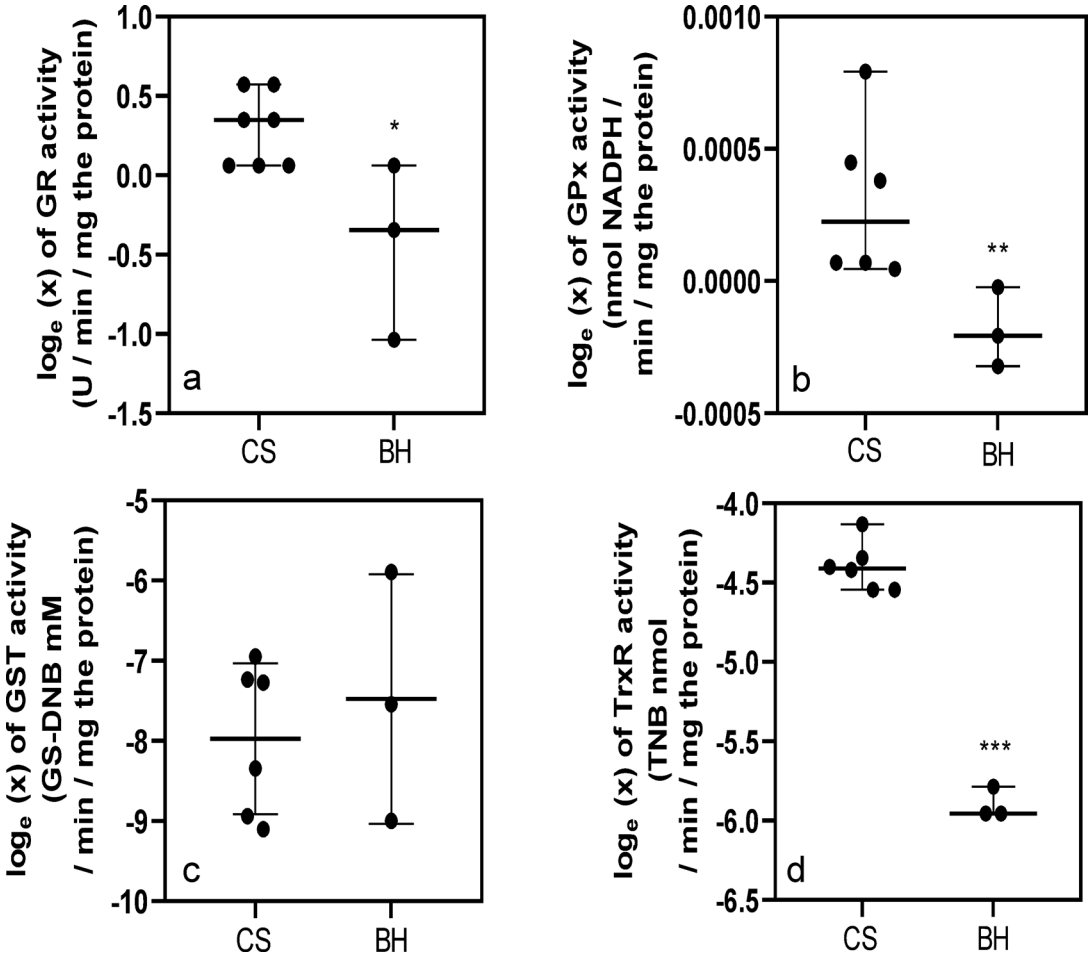

Fig. 2. GR, GPx, GST and TxrR activities in TAA and the AFTA of the patients with BH and CS respectively. *p enzymatic system = 0.04, ** $p = 0.01$ and *** $p = 0.001$.

sense, the oxidative modifications to TE by O_2^- could alter EF assembly. Modifications to the C-terminal domain of TE could affect deposition of TE aggregates into microfibrils. Removal of the C-terminal domain reduces the ability of TE to assemble into EF[35].

Study limitations

Due to the fact that BH syndrome is a rare pathology where the prevalence is less than 1 in 10,000 cases, the recruitment of these patients is scarce, and it is very difficult to collect samples. This may seem a first limitation; however, this is really not so, because some of the results are clear as preliminary findings even if the number of cases is very low. Although several results did not reach a statistically significant difference, the trends are biologically relevant and important given the small "n" of cases. Furthermore, the low number of cases in this study indicates that the participation of OS is a mechanism that deserves attention for possible therapeutic targets. Therefore, since there exist no studies on this condition, and none have focused on elucidating the participation of OS or the presence of a state of the deregulation of redox homeostasis, this topic requires attention to further consider the adjuvant management of patients with antioxidant therapy in possible clinical trials. On the other hand, although the control subjects that were selected for comparison with BH patients were intervened for aortic valve replacement and were therefore not entirely healthy, they underwent the same procedures as the subjects with BH. These control subjects might present alterations in redox homeostasis; however, the point of comparison lies in the presence and absence of BH. Another study limitation was the lack of evaluation of nuclear factor erythroid 2-related factor 2-Keap1 signaling which is a master regulator of the antioxidant enzymatic system Therefore, the exploration of the participation of this system could provide interesting findings in patients with BH.

Conclusions

The results of this preliminary study in this series of three patients with BH suggest a possible loss of redox homeostasis. This loss could be due to the decrease of some enzymes of the enzymatic antioxidant system such as GPx, TrxR, SOD isoforms, peroxidases and catalase, and also to the loss of some antioxidants of the non-enzymatic system such as GSH, thiols, TAC and NO_3^-/NO_2^- ratio, associate to O_2^- increase. In addition, the

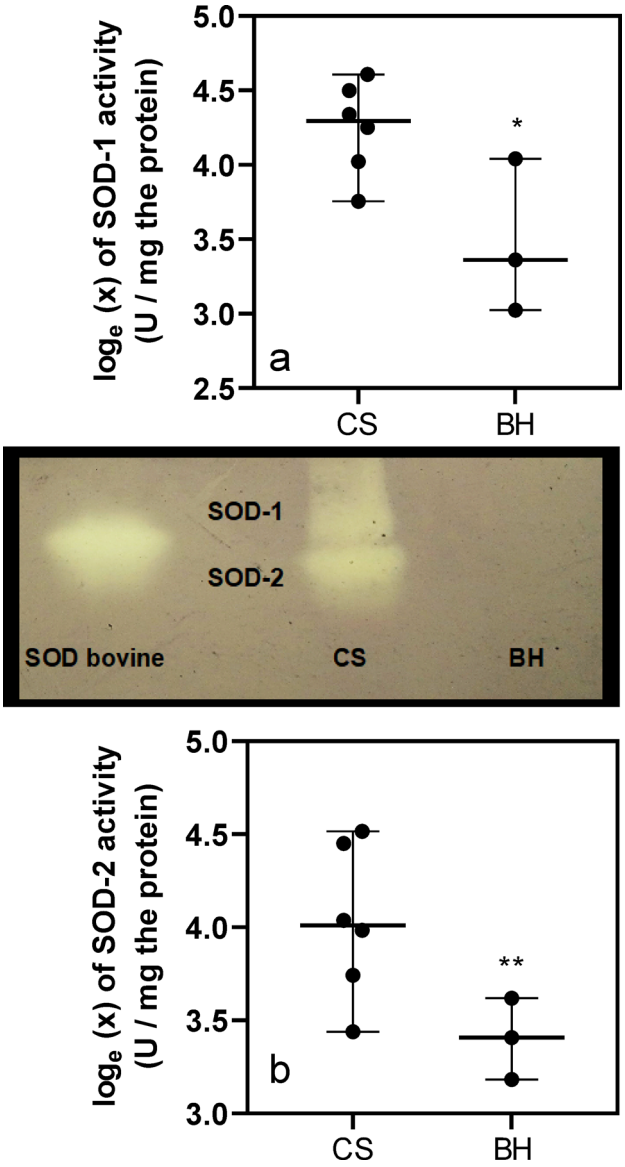

Fig. 3. Activities of the SOD isoforms in TAA and the AFTA of the patients with BH and CS respectively. $^{*}p = 0.02$ and $^{**}p = 0.01$.

decrease in TrxR activity and the concentration of thiol groups could contribute to the alteration and instability of the FBN-2 protein.

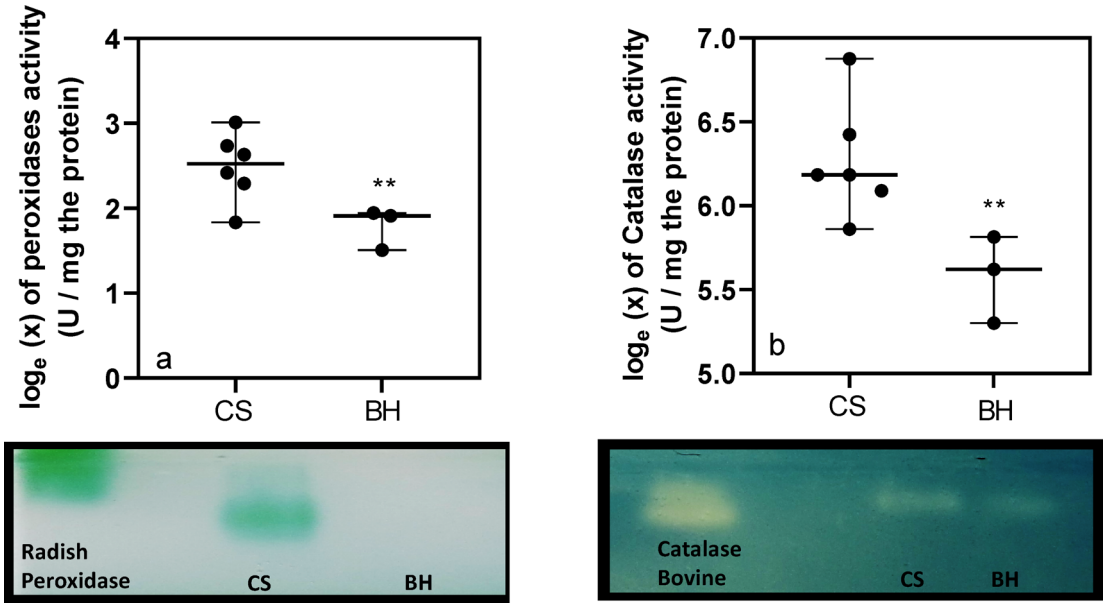

Fig. 4. Activities of the peroxidases and catalase in TAA and the AFTA of the patients with BH and CS respectively. $^*p = 0.02$ and $^{**}p = 0.01$.

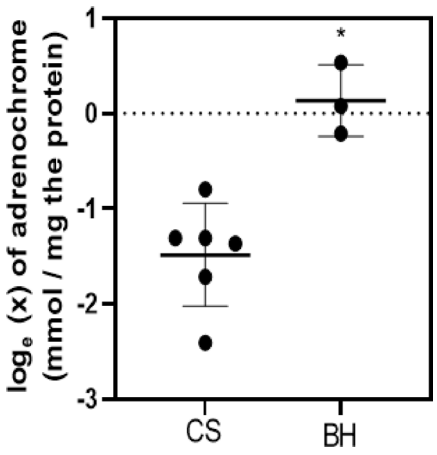

Fig. 5. Indirect detection of the O_2^- by irreversible oxidation of epinephrine to adrenochrome in TAA and the AFTA of the patients with BH and CS respectively, $^*p = 0.02$.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request and available from the María Elena Soto and Israel Pérez-Torres.

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Author contributions

M.E.S. and I.P.-T. Conceptualization, writing, methodology.; L. M.-P. Methodology.; V. G.-L., Writing, review and editing. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethics declarations

Each BH patient signed an informed consent form in accordance with the Helsinki Declaration, as amended by the Congress of Tokyo. Informed consent has been obtained from the patient(s) to publish this paper.

Institutional review board statement

The research protocol was approved by the research and ethics committee of the National Institute of Cardiology Ignacio Chávez (protocol number 23-1366).

Additional information

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