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The Comparison of Apoptosis Anti-apoptosis and Autophagy Markers in Epicardial Tissue in Patients with Coronary Artery Disease and in Patients have Valvular Disease, HOCM, Ascending Aortic Aneurysm without Coronary Artery Disease

Koroner Arter Hastalığı Olan ve Koroner Arter Hastalığı Olmayan Kapak Hastalığı, HOKM, Asendan Aort Anevrizması Olan Hastalarda Epikardiyal Dokuda Apoptoz Anti-apoptoz ve Otofaji Belirteçlerinin Karşılaştırılması

Mesut Karataş[1], Kemal Emrecan Parsova[2], Nurşen Keleş[3], Tanıl Özer[4], Gökhan Alıcı[1]

[1]*University of Health Sciences Türkiye, Kartal Koşuyolu Yüksek İhtisas Training and Research Hospital, Clinic of Cardiology, İstanbul, Türkiye*

[2]*Medicana Ataşehir Hospital, Clinic of Cardiology, İstanbul, Türkiye*

[3]*University of Health Sciences Türkiye, Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital, Clinic of Cardiology, İstanbul, Türkiye*

[4]*University of Health Sciences Türkiye, Kartal Koşuyolu Yüksek İhtisas Training and Research Hospital, Clinic of Cardiovascular Surgery, İstanbul, Türkiye*

ABSTRACT

Background: Apoptosis is a highly programed mechanism that occurs as a part of cell life. Anti-apoptotic and pro-apoptotic proteins regulate this process. Autophagy is a catabolic process that involves the degradation and recycling of aged cytoplasmic components. In this study, we evaluated the expression rates of bax (apoptosis), Bcl-2 (anti-apoptosis), and beclin (autophagy) proteins in epitadial tissue in cardiovascular diseases.

Materials and Methods: Patients with coronary artery by-pass graft (CABG) due to coronary artery disease constituted the patient group. Patients who underwent mitral valve repair (MVR) for severe mitral regurgitation, or who underwent aortic valve replacement (AVR) for severe aortic regurgitation, or who underwent myectomy for hypertrophic obstructive cardiomyopathy (HOCM) or Benthall procedure for ascending aortic aneurysm were included in the study as a control group. Biopsy samples were taken from the epicardial tissues during surgery. Sections taken from the samples were stained with hematoxylin and eosin and examined histopathologically under a light microscope to evaluate inflammation intensity. Samples found suitable were stained immunohistochemically with bronchioloalveolar carcinoma, Bcl-, and Beclin antibodies.

Results: Inflammation in patients with CABG was considerably higher than that in the control group. In patients with CABG with inflammation, Bax expression increased in parallel with inflammation. Bcl-2 expression was significantly increased in patients with MVR, AVR, or myectomy. The highest rate of Beclin expression was observed in HOCM patients. Similar results were obtained in the patient who underwent the Benthall procedure as in the HOCM patient. Beclin expression was also increased in patients who underwent AVR or MVR and showed high Bcl-2 expression.

Conclusion: All of the participants had Bax, Bcl-2, and Beclin expression, albeit at different levels. Inflammation level is determinative for Bax and Beclin expression. In patients with inflammatory CABG, Bax expression increases in parallel with inflammation. In patients with MVR or AVR or myectomy without ischemic heart disease, Bcl-2 expression increased much more than in patients with CABG.

Keywords: Apoptosis, cardiovascular diseases, Bcl-2, Bax, Beclin

Address for Correspondence: Kemal Emrecan Parsova, Medicana Ataşehir Hospital, Clinic of Cardiology, İstanbul, Türkiye

E-mail: emrecanparsova@gmail.com **ORCID ID:** orcid.org/0000-0002-2436-0241

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ÖZ

Amaç: Apoptoz, hücre yaşamının bir parçası olarak ortaya çıkan yüksek düzeyde programlanmış bir mekanizmadır. Anti-apoptotik ve pro-apoptotik proteinler bu süreci düzenler. Otofaji, yaşlanmış sitoplazmik bileşenlerin parçalanması ve geri dönüşümünü içeren katabolik bir süreçtir. Bu çalışmada kardiyovasküler hastalıklarda epikadiyal dokuda Bax (apoptoz), Bcl-2 (anti-apoptoz), Beclin (otofaji) protein ekspresyon oranlarını değerlendirdik.

Gereç ve Yöntemler: Koroner arter hastalığı nedeniyle koroner arter by-pass grefti (KABG) yapılan hastalar hasta grubunu oluşturmuştur. Şiddetli mitral yetersizliği nedeniyle MVR yapılan veya şiddetli aort yetersizliği nedeniyle AVR yapılan veya hipertrofik obstrüktif kardiyomiyopati (HOCM) nedeniyle miyektomi yapılan veya çıkan aort anevrizması nedeniyle Benthall prosedürü uygulanan hastalar kontrol grubu olarak çalışmaya dahil edildi. Ameliyat sırasında epikardiyal dokulardan biyopsi örnekleri alındı. Örneklerden alınan kesitler hematoksilen ve eozin ile boyandı ve inflamasyon yoğunluklarını değerlendirmek için ışık mikroskobu altında histopatolojik olarak incelendi. Uygun bulunan örnekler immünohistokimyasal olarak BAC, Bcl- ve Beclin antikorları ile boyandı.

Bulgular: KABG hastalarında enflamasyon kontrol grubuna göre oldukça yüksekti. Enflamasyonlu KABG hastalarında Bax ekspresyonu enflamasyona paralel olarak artmıştır. MVR veya AVR veya miyektomi yapılan hastalarda Bcl-2 ekspresyonu anlamlı derecede artmıştır. En yüksek Beclin ekspresyonu oranı HOCM hastasında gözlendi. Benthall prosedürü uygulanan hastada da HOCM hastasında olduğu gibi benzer sonuçlar elde edildi. Beclin ekspresyonu AVR veya MVR yapılan ve yüksek Bcl-2 ekspresyonu gösteren hastalarda da artmıştır.

Sonuç: Katılımcıların hepsinde farklı düzeylerde de olsa Bax, Bcl-2 ve Beclin ekspresyonu vardı, enflamasyon düzeyi Bax ve Beclin ekspresyonu için belirleyicidir, enflamatuvar KABG'li hastalarda özellikle Bax ekspresyonu inflamasyona paralel olarak artar, İskemik kalp hastalığı olmayan MVR veya AVR veya miyektomili hastalarda Bcl-2 ekspresyonları KABG'li hastalara göre çok daha fazla artmıştır.

Anahtar Kelimeler: Apoptoz, kardiyovasküler hastalıklar, Bcl-2, Bax, Beclin

Introduction

Apoptosis is a highly programed, energy-dependent mechanism that occurs as a part of cell death and development, tissue turnover, and the immune system without damaging the surrounding tissue (1). Apoptosis is the bridge between pro-death and survival signals, and its outcome is key to cell fate (2). It is characterized by its specific morphology, which includes cell shrinkage, chromatin condensation, DNA fragmentation, membrane blebbing, and formation of apoptotic bodies (1). Data obtained from the examination of human heart tissue demonstrated increased apoptosis in idiopathic dilated cardiomyopathy, ischemic cardiomyopathy, and hypertrophic cardiomyopathy (1). The degree of apoptosis associated with such conditions is usually quite low; however, it is thought that the gradual loss of heart cells over time may contribute to eventual heart failure (1). In addition, the majority of tissues examined were obtained in the last stage of the disease, and it has been suggested that apoptosis may play a role in the transition from mild hypertrophy to end-stage heart failure (2). Apoptosis is regulated by the complex interplay of numerous prosurvival and prodeath signals. In particular, the Bcl-2 protein family consists of both anti-apoptotic (Bcl-2, Bcl-xl) and pro-apoptotic (Bax, Bid) proteins and exerts its effects by altering the integrity of the mitochondrial membrane and by releasing apoptotic intermembrane proteins (2).

Autophagy is a catabolic process involving the degradation and recycling of aged cytoplasmic components, such as long-lived proteins and organelles, with the aid of lysosomes (3). Under normal conditions, it is responsible for maintaining homeostasis. In nutrient-deprived cells, autophagy is a cell-survival mechanism but also mediates cell death under certain conditions (3). It has various physiological and pathophysiological roles such as adaptation to nutrient deprivation, protein intracellular clearance, growth, anti-aging, elimination of microorganisms, cell death, tumor suppression, and antigen presentation (4). Autophagy in the heart appears to be upregulated in conditions such as ischemia -reperfusion and heart failure (3). It is unclear whether autophagy is beneficial or dangerous in the heart because it appears to modulate cell viability and death. The presence of autophagic vacuoles in dying cells can be assessed in one of two ways: cells activate autophagy for survival or as part of cell death (3). In a cell with insufficient autophagic activity, aged proteins and defective organelles accumulate and eventually apoptotic cell death occurs (4). Conversely, if autophagy degrades proteins and organelles beyond a certain threshold, autophagic cell death will occur, especially in cells with an inadequate apoptotic response (4). It can be concluded that the crossover mechanism occurs between the two mechanisms and that the balance between autophagy and apoptosis maintains homeostasis. Autophagy is mainly controlled by autophagy-related genes that regulate autophagosome formation (3). Beclin-1 is required for the vesicle nucleation stage of autophagy (3).

In this study, we evaluated the expression rates of bax (apoptosis), Bcl-2 (anti-apoptosis), and beclin (autophagy) protein in epitadial tissue in patients with coronary artery

by-pass graft (CABG) or mitral valve replacement (MVR) or aortic valve replacement (AVR) or Benthall procedure or myectomy.

Materials and Methods

Study Design and Study Population

The study was conducted between 2016 and 2018 at the University of Health Sciences Türkiye Kartal Koşuyolu High Specialization Training and Research Hospital. In the study, patients who underwent CABG because of obstructive coronary artery disease constituted the patient group. The patients were evaluated in the cardiology outpatient clinic because of chest pain, and coronary angiography was performed according to the results of the effort test or myocardial perfusion test. All patients had normal troponin levels. Patients with normal coronary arteries detected on preoperative coronary angiography who underwent MVR for severe mitral regurgitation, or who underwent AVR for severe aortic regurgitation, or who underwent myectomy for hypertrophic obstructive cardiomyopathy (HOCM) or Benthall procedure for ascending aortic aneurysm were included in the study as a control group. The left ventricular systolic functions of the patients were preserved both preoperatively and postoperatively (ejection fraction >50%). One of the patients with severe mitral regurgitation had mitral valve prolapse, whereas the others had rheumatic valve disease. All patients with advanced aortic regurgitation have degenerative valve disease. The study was carried out with the approval of the local ethics committee (2023/06/683). All patients were informed in detail about biopsy procedures, surgical procedures to be performed, all complications that may occur during and after surgical procedures, the follow-up period, and patient consent forms.

Histopathological Examination

Biopsy samples were taken from the epicardial tissues of the patients during surgery. Formalin-fixed paraffin blocks were prepared from the biopsy samples. Sections of 4-5 microns taken from the samples were stained with hematoxylin and eosin (H&E) and examined histopathologically under a Nikon Eclipse E600 light microscope. Inflammation intensities in the patients were assessed by considering the number of inflammatory cells entering the high magnification field in light microscopic examination and were classified as none (score 0), mild (score 1), moderate (score 2) and severe (score 3) (Figure 1). Samples found suitable because of histopathological examination were stained immunohistochemically with BAC, Bcl- and Beclin antibodies.

Immunohistochemical Examination

Samples were stained immunohistochemically using the ABC method for Bax, Bcl- and Beclin antibodies. Sections of 4 µm thickness taken on adhesive slides (Surgipath, X-tra Adhesive Microslides, Illinois, USA) and kept in an oven at 56 °C for 12 h. The sections were dried in xylene for 30 min and kept in 100% (absolute), 96%, 90%, and 80% ethyl alcohol for 15 min for dehydration. The sections were washed with water and distilled water. To block endogenous peroxidase activity, 3% H_2O_2 prepared in methanol was added for 10 min. Sections were washed

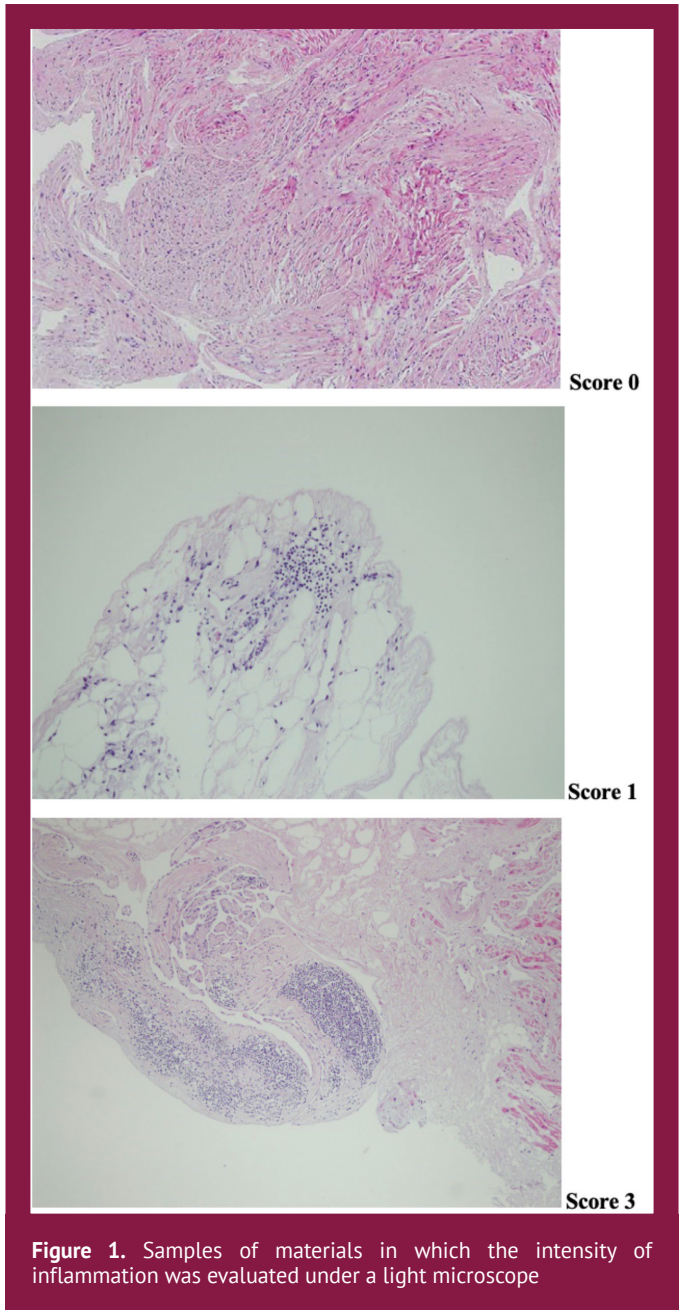

Figure 1. Samples of materials in which the intensity of inflammation was evaluated under a light microscope

with phosphate-buffered saline (PBS) (pH =7.60) using the spray method. To reveal the antigenic structures masked in the tissue due to formalin fixation and paraffin blocking, the sections to which both antibodies will be applied are in antigen retrieval solution (0.01M sodium citrate buffer, pH =6.00), medium for the first 10 min, and high for the last 5 min. It was processed in a microwave oven for 15 min. Subsequently, the sections were incubated at room temperature for 30 min and washed three times with distilled water followed by PBS. The Zymed Histostain Plus Broad Spectrum kit (Lot 1385070, Zymed laboratories Inc., Carlsbad, CA, USA) was used for all antibodies. Sections were kept in non-immune blocking serum for 5-10 min. Then, iNOS (polyclonal antibody RB -1605-P1; Neo Marker, Fremont, CA, USA), HSP27 (monoclonal antibody clone G3.1, MS-101- R7, Neo Marker, Fremont, CA, USA), and HSP 60 (monoclonal antibody clone LK1, 101-R7, Neo Marker, Fremont, CA, USA) at +4 ^{0}C for 18-20 hours. After reaching room temperature, the sections were washed with PBS for 5 min by spraying and dried, and then biotin-bound (secondary) antibody was applied for 20-30 min. After the sections were washed again with PBS and dried, alkaline phosphatase conjugated with streptavidin was applied and left for 20-30 min. Subsequently, the sections were washed with PBS and dried. The mixture was incubated with AEC chromogen solution (Zymed Laboratories, San. Francisco, Calif, USA, Lot: 00-0027) for approximately 10-15 min to provide color rendering. Sections were run thrice in distilled water. It was kept in Mayer's hematoxylin for 30 s for background staining. Sections were washed first in tap water and then in distilled water. The slide was then closed using a water-based sealant (Large volume vision mount, Lot:UG14124, labvision Fremont, CA, USA).

Immunohistochemical Evaluation

Cytoplasmic and nuclear staining of myocyte cells in epicardial fat samples were considered positive for Bax, Bcl-2, and Beclin expression. Each sample considered positive was graded according to the expression intensity. In addition, other cells in the connective tissue that showed positivity were not recorded.

Statistical Analysis

Descriptive statistics were used to summarize the case data by diagnostic group. Continuous variables; were evaluated using mean, standard deviation, minimum and maximum values, and ratio and percentage values for categorical variables. One-way analysis of variance was used to compare the numerical variables with more than two groups that were normally distributed, and the Kruskal-Wallis test was used for the variables that were not normally distributed. Comparisons between the two groups were made using Student's t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Categorical variables were analyzed using crosstalk statistics (chi-square test, Fisher test). In crosstalk statistics, when the hypothesis of the test to be used in the analysis was not met, the necessary transformations (group aggregation) were made and the data were reanalyzed. Statistical significance was defined as a p value of 0.05. IBM SPSS Statistics version 12.0 (SPSS Inc, Chicago USA) was used in the analysis.

Results

The study included 28-CABG, 4 MVR, 3 AVR, 1 myectomy, and 1 Benthall procedure patients. There were 5 women and 23 men aged between 44 and 75 years in the patient group, whereas the control group included 3 women and 7 men aged between 24 and 68 years. The comorbid diseases of the patients are shown in Table 1.

In the examination of sections obtained from biopsy samples by H&E staining, it is noteworthy that the inflammation in patients diagnosed with ischemic heart disease and therefore undergoing CABG was considerably higher than that in the control group (Table 2).

Bax, Bcl-2, and Beclin expressions in the patient and control groups are shown in Table 3.

It was observed that Bax, Bcl-2, and Beclin expression was observed in all the patient and control groups, albeit at different levels. In patients with CABG with inflammation, it was observed that Bax expression increased in parallel with inflammation. Bcl-2 expression was significantly increased in patients with MVR, AVR, or myectomy without ischemic heart disease compared with patients with CABG (Figure 2). Bax expression, which was detected at a high rate in patients with CABG, suggested that ischemia also triggers apoptosis. In addition to high Bax expression in CABG patients, Bcl-2 expression was increased in these patients. The highest rate of Beclin expression was observed in HOCM patients (Figure 3). Similar results were obtained in the patient who underwent the Benthall procedure as in the HOCM patient (Figure 4). Beclin expression was also increased in patients who underwent AVR or MVR and showed high Bcl-2 expression.

No myocardial infarction, stroke, or death occurred during the in-hospital follow-up of the patients.

Discussion

In the patient group, there were patients with pre-operative and post-operative LVEF >50% who underwent diagnostic angiography for suspected coronary artery

Table 1. The intensity of inflammation in the study population. The first 28 patients consisted of CABG patients. Biopsy samples were obtained from the right atrium (RA) appendix and the periventricular region adjacent to the left anterior descending artery (LAD). Control group patients are shown in red

Patient	Sex	RA appendix	LAD periventricular
1	M	0	0
2	F	0	1
3	M	0	0
4	M	0	0
5	M	1	0
6	M	3	0
7	M	0	0
8	M	0	1
9	M	0	0
10	M	0	0
11	M	0	0
12	M	1	0
13	M	0	0
14	M	3	0
15	M	0	0
16	M	3	0
17	M	3	0
18	M	1	0
19	M	0	0
20	M	0	0
21	M	0	0
22	M	0	0
23	F	0	0
24	M	3	1
25	M	0	0
26	M	0	0
27	M	3	0
28	F	3	0
29	F	0	0
30	F	0	
31	F	0	
32	M	0	
33	M	0	
34	F	0	
35	M	0	
36	M	0	
37	F	0	
38	M	0	

F: Female, M: Male, LAD: Left anterior descending artery, RA: Right atrium

disease and whose coronary artery disease was confirmed; therefore, CABG was performed. The control group included patients with MVR, AVR, Benthall procedure, or myectomy with normal or plaque coronary arteries on preoperative coronary angiography and pre-operative and post-operative LVEF >50%. The expression rates of Bax (apoptosis), Bcl-2 (antiapoptosis), and Beclin (autophagy) proteins were measured in epicardial tissue samples taken from the patients during surgery.

Several proteins can initiate apoptosis in mitochondria. These proteins neutralize cytochrome c and endogenous apoptosis inhibitors. The choice between cell death or survival is determined by mitochondrial permeability, which is controlled by more than 20 proteins, the prototype of which is Bcl-2. Multiple sensors are activated when cells are deprived of growth factors and other life signals or when they encounter DNA-damaging factors or accumulate large amounts of misfolded proteins. These sensors are members of the Bcl-2 family called "BH3 proteins". BH3 proteins activate two proapoptotic members of the family, Bax and Bak. These dimerize and enter the mitochondrial membrane and form channels through which cytochrome c and other mitochondrial proteins can pass into the cytosol. Again, the same sensors inhibit anti-apoptotic molecules called Bcl-2 and Bcl-xL, making it easier for mitochondrial proteins to pass into the cytosol. Cytochrome c with other cofactors activates caspase-9. Other proteins that pass from mitochondria to the cytosol block caspase antagonists, which act as physiological inhibitors of apoptosis. The net result of all these interactions is the activation of the caspase cascade, which results in fragmentation of the cell nucleus. However, when cells continue to receive growth factors and other life signals, they synthesize anti-apoptotic members of the Bcl-2 family. One of the two main types is Bcl-2 itself, and the other is Bcl-xL. Because these proteins antagonize Bax and Bak proteins, they limit the exit of pro-apoptotic proteins from mitochondria. Cells deprived of growth factors not only activate the Bax and Bak proteins. They also decreased the levels of Bcl-2 and BclxL, causing the balance to shift more toward cell death. The mitochondrial pathway appears to be responsible for apoptosis in most cases, as described later (5-9).

Detection of Bax > Beclin in patients undergoing CABG for myocardial ischemia shows that ischemia causes severe death in myocytes not only by necrosis but also by apoptosis and autophagy (even if LVEF is preserved). In the process of necrosis, which starts with cell membrane damage after the prolongation of the ischemia period and the increase in the severity of the existing ischemia and therefore the decrease in intracellular energy, the calcium

entering the cell can directly stimulate the caspases and initiate apoptosis via the intrinsic way through the mitochondria.

Only 30% of the cells in the heart wall are myocytes, but because oxygen consumption is high in these cells, ischemia and apoptotic processes triggered by ischemia should be expected to develop predominantly in these cells. It was thought that the increase in apoptosis determined by the increase in Bax in the heart wall was observed in ischemic myocytes. In this process, it can be thought that the general lack of nutrients in the cells of the heart wall stimulates autophagy through Beclin.

The high expression of Bcl-2 in the control group patients with normal coronary angiography and preserved LVEF indicates that the cells escaped death and that the expression of claims was significantly increased in these patients, the adaptation capacity of the cells to the physical stresses causing the surgery was exceeded, myocyte death started via autophagy, and the heart began to dilate may be an indicator.

In the patient group without ischemic findings in the heart wall, heart wall cells showed Bcl-2 expression and Bax expression was low. This suggested that there was no intense stress that could cause cell death in the heart wall cells. However, as in heart valve diseases, the strain of heart

Table 2. Bax, Bcl-2, and Beclin expressions in the patient group

	Expressio	Standard deviation		Expressio	Standard deviation		Expressio	Standard deviation
1A BAX	28.787	6.785	11A-BAX	32.662	9.003	21A BAX	23.631	5.452
1A BCL2	34.196	5.84	11A BCL2	28.611	8.473	21 ABCL2	29.875	4.267
1A BECLIN	30.026	8.78	11A-BECLIN	37.07	10.612	21A-BECLIN	22.345	4.342
2A BAX	28.033	6.819	12A-BAX	34.718	6.894	22A-BAX	23.761	5.592
2A BCL2	24.262	5.317	12A BCL2	36.136	6.319	22A BCL2	30.067	4.342
2A BECLIN	29	8.025	12A-BECLIN	24.289	4.784	22A-BECLIN	23.267	4.769
3A BAX	33.25	9.101	13A BAX	32.382	7.526	23A BAX	24.346	6.415
3A BCL2	25	7.841	13A BCL2	30.487	4.658	23A BCL2	33.917	4.353
3A BECLIN	41.687	13.905	13A-BECLIN	31.585	9.005	23A BECLIN	25.296	4.741
4A BAX	33	8.613	14A BAX	27.113	7.276	24A-BAX	39.462	9.995
4A BCL2	36.899	7.229	14A BCL2	27.833	7.297	24A BCL2	56.898	9.421
4A BECLIN	28	7.563	14A-BECLIN	26.976	8.273	24A-BECLIN	31.193	8.564
5A BAX	27.816	5.341	15A-BAX	25.352	5.591	25A BAX	61.252	18.715
5A BCL2	22	7	15A BCL2	41.466	9.609	25A BCL2	73.388	13.326
5A BECLIN	37.099	9.29	15A-BECLIN	25.698	6.042	25A BECLIN	47.255	13.88
6A BAX	21	4.823	17A-BAX	43.059	17.01	27A BAX	42.72	12.13
6A BCL2	22.895	6.59	17A BCL2	28.641	9.743	27A BCL2	58.174	14.496
6A BECLIN	28	7.92	17A-BECLIN	29.474	6.654	27A BECLIN	41.762	12.86
7A BAX	35.745	9.885	18A BAX	18.314	7.338	28A BAX	37.403	10.939
7A BCL2	37	7.431	18A BCL2	15.471	6.621	28A BCL2	30.212	9.103
7A BECLIN	41.249	13.402	18A-BECLIN	11.306	4.977	28A-BECLIN	29.498	8.084
8A BAX	24	6.696	19A BAX	23.061	8.274	29A BAX	26.079	10.861
8A BCL2	22.531	6.094	19A BCL2	21.733	4.431	29A BCL2	17.725	8.95
8A BECLIN	23	5.702	19A-BECLIN	15.087	4.033	29A BECLIN	18.822	6.105
9A BAX	33.934	5.529	20A BAX	29.546	6.177	30A-BAX	11.206	4.033
9A BCL2	22	6.732	20A BCL2	19.132	6.139	30A BCL2	14.614	4.469
9A BECLIN	27.259	8.221	20A-BECLIN	17.13	6.445	30A-BECLIN	13.68	4.522
10A-BAX	26	7.738						
10A BCL2	37.693	6.33						
10A-BECLIN	25	6.189						

Table 3. Bax, Bcl-2, and Beclin expressions in the control group

	Expressio	Standard deviation		Expressio	Standard deviation
16A BAX	34.945	9.599	34A BAX	38.095	11.374
16A BCL2	32.942	9.551	34A BCL2	46.135	16.556
16A BECLIN	28.902	9.883	34A BECLIN	32.812	9.026
26 A BAX	33.197	7.556	35A BAX	35.915	8.186
26A BCL2	28.629	7.789	35A BCL2	54.032	14.249
26A-BECLIN	29.521	6.189	35A BECLIN	44.192	13.468
31A BAX	13.504	4.69	36A-BAX	36.423	8.448
31A BCL2	13.574	4.773	36A BCL2	57.451	11.506
31A-BECLIN	13.008	6.278	36A-BECLIN	42.602	18.452
32A-BAX	24.954	7.894	37A BAX	32.286	7.868
32A BCL2	50.942	16.65	37A BCL2	33.153	8.31
32A-BECLIN	41.231	9.87	37A-BECLIN	30.909	7.004
33A BAX	27.555	4.171	38A-BAX	28.84	6.221
33A BCL2	34.987	6.758	38A BCL2	34.221	7.776
33A-BECLIN	33.374	14.932	38A-BECLIN	26.788	5.002

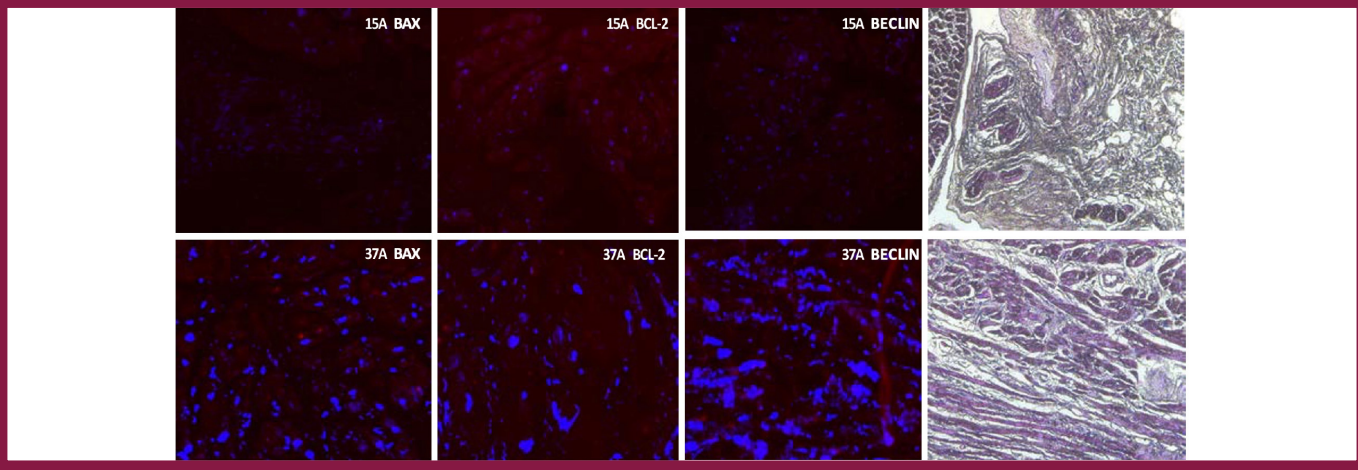

Figure 2. Outcomes of AVR patients (anti-apoptosis > apoptosis ⩾ autophagy)
AVR: Aortic valve replacement

wall cells and high energy needs lead to the depletion of energy sources in the environment, suggesting that autophagy is stimulated through nutrient deficiency. Here, the energy source includes oxygen in the short term, but glucose and other substances can be converted into energy in the long term. It can be thought that the heart wall cells use substances that they can convert into energy in the cell after oxygen deficiency and go to autophagy in the final stage. Similarly, it can be thought that autophagy develops after the use of oxygen and then substances that can be converted into energy in the ischemic heart muscle.

Study Limitations

This study was conducted with a small group of patients. Not all valve diseases were evaluated in this study. Mixed serious valve diseases were not evaluated together. There is no long-term follow-up of patients after discharge.

Conclusion

All of the participants had Bax, Bcl-2, and Beclin expression, albeit at different levels. Inflammation level is determinative for Bax and Beclin expression. In patients with inflammatory CABG, Bax expression increases in

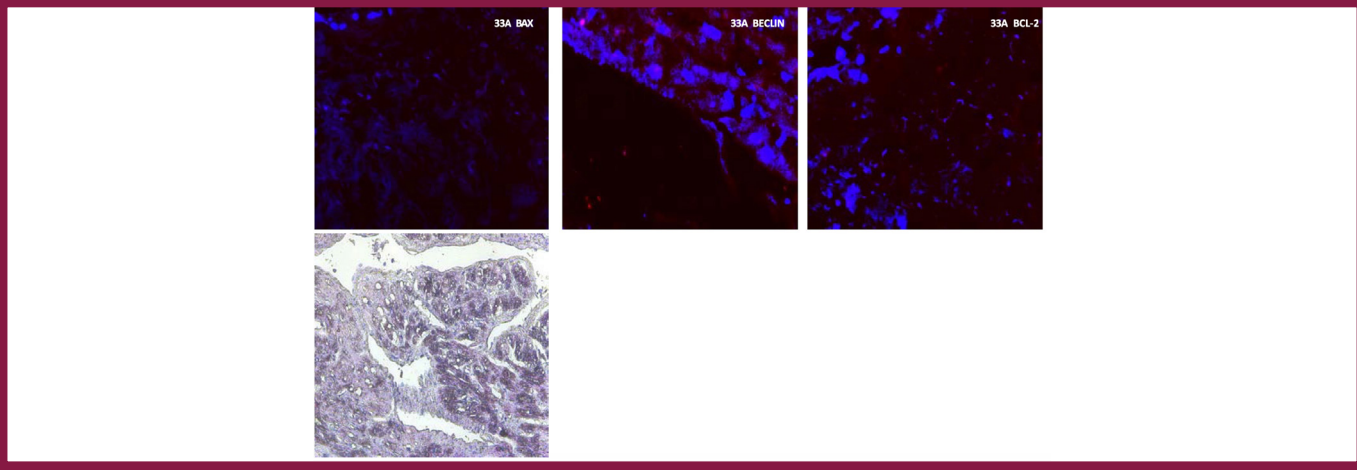

Figure 3. HOCM (anti-apoptosis > autophagy > apoptosis)
HOCM: Hypertrophic obstructive cardiomyopathy

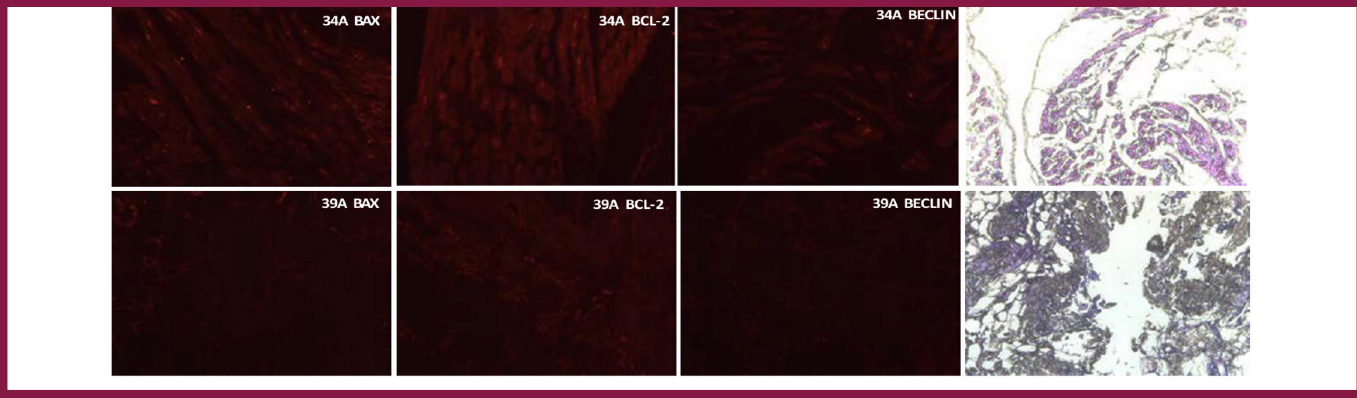

Figure 4. Benthall procedure (anti-apoptosis > apoptosis ⩾ autophagy)

parallel with inflammation. In patients with MVR or AVR or myectomy without ischemic heart disease, Bcl-2 expression increased much more than in patients with CABG. The increased rate of inflammation in patients with CABG possibly developed secondary to ischemic necrosis, and the high rate of Bax in the same patient group suggested that ischemia triggered not only coagulation necrosis but also severe apoptosis in coronary artery patients who underwent CABG. Because coronary blood flow will be restored with bypass grafts in the patient group undergoing CABG, free radical damage and apoptosis will continue in myocytes that are in ischemia and encounter oxygenated blood again, which will cause high Bax expression. Considering the increased Bcl-2 expression in patients with CABG in addition to increased Bax expression, Bcl-2 expression represents myocytes that resist death, it was understood that high Bax expression in patients with CABG was directly caused by ischemia, considering the high Bcl-2 levels in the control groups without ischemia. The increase in Beclin expression in patients who had AVR or MVR showed high Bcl-2 expression, indicating that the adaptation capacity of myocytes against increased stress was reached and that myocytes died by autophagy without ischemia. In patients with HOCM, the highest rate of Beclin expression in genetically thickened myocytes leads to the death of myocytes via autophagy when increased protein synthesis in the cell exceeds the storage capacity of the cells, even in the absence of ischemia and physical stress. Patients who underwent ascending aortic grafting for an ascending aortic aneurysm had similar results to those of HOCM patients. In line with these results, it was understood that ischemia is the strongest death herald for myocytes, causing coagulation necrosis by hypoxia, as well as triggering apoptosis at a high rate. We have shown that it is not caused by necrosis or

apoptosis, but rather by autophagy. This can be used in the timing of surgery, especially in patients with moderate-to-severe valve disease without coronary artery disease.

Ethics

Ethics Committee Approval: The study was carried out with the approval of the local ethics committee (2023/06/683).

Informed Consent: All patients were informed in detail about biopsy procedures, surgical procedures to be performed, all complications that may occur during and after surgical procedures, the follow-up period, and patient consent forms.

Peer-review: Externally and internally peer reviewed.

Authorship Contributions

Concept: M.K., G.A., Design: M.K., G.A., Data Collection or Processing: M.K., T.Ö., G.A., Analysis or Interpretation: M.K., N.K., T.Ö., G.A., Literature Search: M.K., K.E.P., N.K., Writing: M.K., K.E.P., N.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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