Alternative causes of bioreaction to prosthetic heart valves: three cases with pannus formation

Protez kalp kapaklarında gelişen biyoreaksiyonun alternatif sebepleri: Pannuslu üç olgu

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Summary– Pannus formation is an infrequent but serious complication of prosthetic heart valve surgery. The cause of pannus is recognized as a bioreaction to the prostheses; histological investigations have shown that pannus comprises collagen and elastic tissues containing endothelial cells, chronic inflammatory cells, and myofibroblasts. However, the detailed mechanism of its formation has not been fully demonstrated. We aimed to evaluate the potential role of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) in the pathogenesis of pannus formation in three patients with mechanical prosthetic heart valves. Pannus specimens removed from the prostheses were fixed in 10% neutral-buffered formalin for 24 hours after surgical removal and paraffin-embedded using standard procedures. Serial sections were cut at 4 μm for immunohistochemistry analysis. Hematoxylin and eosin (HE) was used in the histological analysis. VEGF and MMP-2 were studied in the immunohistochemistry analysis. Three patients with mechanical prosthetic obstruction due to pannus overgrowth underwent redo valve surgery. In the first and second patients, the mitral prosthesis was explanted along with the pannus overgrowth. The third patient had both aortic and mitral prostheses; the aortic prosthesis was explanted with obstructive pannus formation, whereas the mitral valve was spared with excision of the nonobstructive pannus. The immunohistochemical study demonstrated the expressions of MMP-2 and VEGF in all of the pannus specimens acquired from these cases. VEGF and MMP-2 may play a role in the mechanism of pannus formation as the elements of the chronic active inflammatory process.

Özet– Pannus oluşumu protez kapak cerrahisinin nadir ama ciddi bir komplikasyonudur. Pannusun oluşum nedeni protez kapağa karşı gelişen biyoreaksiyon olarak kabul edilmektedir. Yapılan histopatolojik araştırmalar pannus dokusunda endotel hücreleri içeren kolajen ve elastik dokuların, kronik iltihabi hücrelerin ve miyofibroblastların varlığını göstermiştir. Fakat pannus oluşumunun detaylı mekanizması net olarak gösterilememiştir. Bu olgu sunumunda, mekanik protez kalp kapağında pannus gelişen üç hastada vasküler endotelyal büyüme faktörünün (VEGF) ve matriks metalloproteinaz (MMP) 2'nin pannus oluşumundaki potansiyel rolü araştırıldı. Tıkayıcı pannus oluşumu nedeniyle tekrar kapak cerrahisi uygulanan hastalardan çıkarılan pannus doku örnekleri 24 saat süresince %10'luk tamponlanmış nötral formalin solüsyonunda sabitleştirildi. Standart yöntemle parafin blokları hazırlandı. Bu bloklardan immünohistokimyasal analiz için 4 mikrometre kalınlığında kesitler alındı ve hematoksilen ve eosin (HE) ile boyandı. İmmünohistokimyasal analizde VEGF ve MMP-2 araştırıldı. Tıkayıcı pannus nedeniyle tekrar kapak cerrahisi uygulanan üç hastadan, birinci ve ikinci hastada pannus dokusu gelişen mitral kapak çıkarıldı. Hem aorta hem de mitral pozisyonunda protez kapağı olan üçüncü hastada ise tıkayıcı pannus gelişen aort kapak çıkarılırken, mitral protez kapağındaki tıkanıklık yaratmayan pannus dokusu kesilerek kapak korundu. Her üç olguda alınan pannus doku örneklerinden yapılan immünohistokimyasal analizde MMP-2 ve VEGF ekspresyonu gösterildi. Kronik aktif iltihabi süreçte yer alan VEGF ve MMP-2 protez kapakta pannus gelişiminde rol oynayabilir.

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Prosthetic valve dysfunction (PVD) due to pannus formation is an infrequent but serious complication. The cause of pannus is generally recognized as a bioreaction to the prosthesis;[1] however, the detailed mechanism of its formation has not yet been fully demonstrated. Herein, we aimed to evaluate the potential role of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) in the pathogenesis of pannus formation in prosthetic heart valves.

Abbreviations:

2D TEE	*Two-dimensional transesophageal echocardiography*
AVR	*Aortic valve replacement*
INR	*International normalized ratio*
MMP-2	*Matrix metalloproteinase-2*
MVA	*Mitral valve area*
MVR	*Mitral valve replacement*
RT-3D TEE	*Real-time three-dimensional transesophageal echocardiography*
TTE	*Transthoracic echocardiography*
VEGF	*Vascular endothelial growth factor*

We present three cases who underwent redo valve surgery due to obstructive pannus pathology; the explanted materials were studied in immunohistochemical analysis (Fig. 1).

CASE REPORT

The first case was a 40-year-old man who underwent mitral valve replacement (MVR) (St. Jude Medical, 27 mm) 14 years ago due to rheumatic heart disease. He had exertional dyspnea on admission with an international normalized ratio (INR) of 2.7. ECG showed normal sinus rhythm. Transthoracic echocardiography (TTE) revealed a mean mitral transvalvular gradient of 10 mmHg and a mitral valve area (MVA) of 1.5 cm². Two-dimensional transesophageal echocardiography (2D TEE) and subsequent real-time three-dimensional transesophageal echocardiography

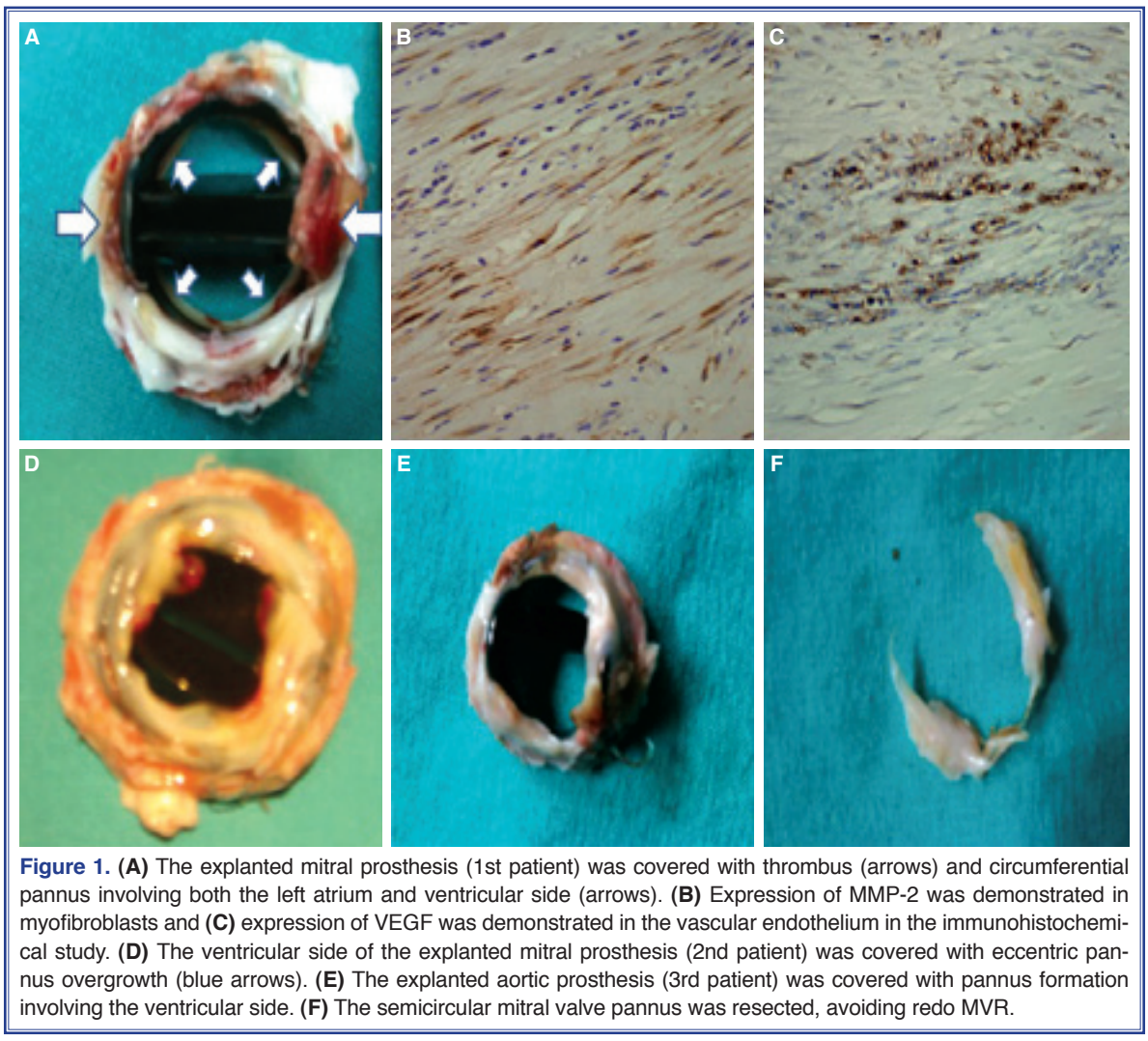

Figure 1. **(A)** The explanted mitral prosthesis (1st patient) was covered with thrombus (arrows) and circumferential pannus involving both the left atrium and ventricular side (arrows). **(B)** Expression of MMP-2 was demonstrated in myofibroblasts and **(C)** expression of VEGF was demonstrated in the vascular endothelium in the immunohistochemical study. **(D)** The ventricular side of the explanted mitral prosthesis (2nd patient) was covered with eccentric pannus overgrowth (blue arrows). **(E)** The explanted aortic prosthesis (3rd patient) was covered with pannus formation involving the ventricular side. **(F)** The semicircular mitral valve pannus was resected, avoiding redo MVR.

(RT-3D TEE) demonstrated a mass, suspected as pannus overgrowth, narrowing circumferentially the inflow and outflow aspects of the prosthesis by extending into the ventricular sides of the mitral annulus, with a small thrombi located on one of the hinges. The patient underwent redo MVR (St. Jude Medical, 27 mm). The explanted valve was covered with thrombus and circumferential pannus involving both the left atrium and ventricular side (Fig. 1a). Expressions of MMP-2 in myofibroblasts (Fig. 1b) and VEGF (Fig. 1c) in vascular endothelium were demonstrated in the immunohistochemical study.

The second case was a 47-year-old woman who underwent MVR with a 27-mm St. Jude Medical prosthesis 12 years ago and suffered cerebrovascular disease five years ago due to prosthetic valve thrombosis. She presented with dyspnea (New York Heart Association [NYHA] class III). On admission, INR was 2.9. TTE revealed a mean mitral transvalvular gradient of 12 mmHg and a MVA of 1.4 cm². 2D TEE and RT-3D TEE suggested pannus formation on the ventricular side of the prosthesis (Fig. 2). The patient underwent redo valve surgery, and the presence of pannus formation was confirmed (Fig. 1d). The immunohistochemical study of the explanted valve showed the expression of MMP-2 and VEGF, as demonstrated in the first patient.

The third case was a 50-year-old woman who underwent aortic valve replacement (AVR) (St. Jude Medical, 19 mm) and MVR (St. Jude Medical, 27 mm) 13 years ago due to rheumatic heart disease. She had dyspnea (Class III) on admission; TTE revealed increased transprosthetic mean gradients on both mitral (mean gradient: 9 mmHg) and aortic (mean gradient: 45 mmHg) prostheses. INR was 2.5. 2D TEE and RT-3D TEE findings were indicative of potential pannus formation on the atrial side of the mitral prosthesis and the ventricular side of the aortic prosthesis. The patient underwent elective valve surgery; the aortic prosthesis was explanted along with pannus ingrowth (Fig. 1e) and redo AVR was performed, whereas the semicircular pannus formation on the mitral prosthesis was removed, avoiding MVR (Fig. 1f). The expressions of MMP-2 and VEGF were both identified in the immunohistochemical study of the mitral and aortic pannus, as shown in the previous two cases.

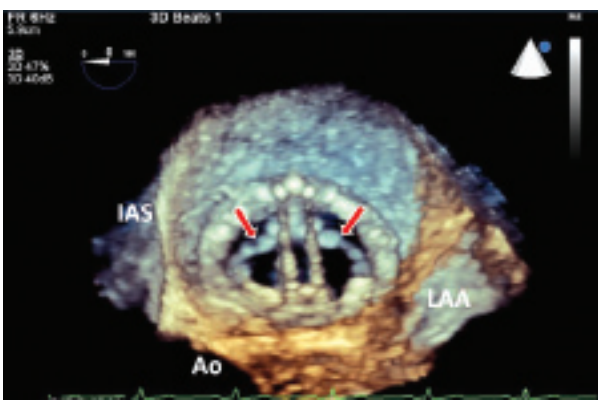

Figure 2. Real-time 3-dimensional transesophageal echocardiography demonstrates pannus formation (see arrows) on the ventricular side of the mitral prosthesis in the 2nd patient. Ao: Eortic; IAS: Interatrial septum; LAA: Left atrial appendage.

Specimen preparation

The tissue samples were fixed in 10% neutral-buffered formalin for 24 hours within 1 hour after surgical removal and were paraffin-embedded using standard procedures. Serial sections were cut at 4 μm for immunohistochemistry analysis. Hematoxylin and eosin (HE) was used in the histological analysis. VEGF (for endothelial cells) and MMP-2 (for demonstration of cytokines secreted from macrophages, leukocytes, fibroblasts, and myofibroblasts) were studied in the immunohistochemistry analysis.

DISCUSSION

Prosthetic valve obstruction is a life-threatening complication and may occur due to thrombosis, patient-prosthesis mismatch or pannus overgrowth.[2] All patients receiving prosthetic valve replacement have a risk of periannular intimal thickening. Pannus formation, which is an overgrowth of fibrous tissue, is an inflammatory reaction against a foreign body that blocks the disc by invading the valve orifice. The pannus formation after prosthetic valve replacement may be associated with a process of periannular tissue healing via the expression of transforming growth factor-beta (TGF-b). Histological investigations have shown that pannus comprises collagen and elastic tissues containing endothelial cells, chronic inflammatory cells, and myofibroblasts.[1,3] The expression of both MMP-2 and VEGF, demonstrated in the pannus of the explanted valves in these three patients, is reported in the literature for the first time.

Any alteration in the synthesis and breakdown of the extracellular matrix is important in tissue remodeling during inflammation and wound healing. The degradation of the extracellular matrix components is regulated by a cascade of MMPs.[4] MMPs play a central role in the regulation of multiple cellular functions such as cell proliferation, adhesion, migration, differentiation, angiogenesis, and apoptosis.[5]

VEGF was the other target of our study, which was studied previously in the synovial joints of rheumatoid arthritis patients but not in the pannus formation of prosthetic valves. VEGF-C overexpression by fibroblasts stimulates multiple biologic processes known to impact wound healing, such as collagen constriction, capillary sprouting, and endothelial progenitor cell invasion and migration through the extracellular matrix.[6]

Although the presence of MMP-2 and VEGF was demonstrated in pannus overgrowth in our three patients, this cannot be generalized to all patients with prosthetic valves, necessitating further research. Histological and immunohistochemical investigations of such molecules associated with tissue remodelling in large-scale studies may highlight the underlying mechanisms of pannus formation.

In conclusion, VEGF, which provides migration of smooth muscle cells and extracellular matrix, and MMP-2, which degrades the extracellular matrix in the remodelling process, may play a role in the mechanism of pannus formation as the elements of the chronic active process.

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Key words: Aortic valve; heart valve diseases; heart valve prosthesis; matrix metalloproteinases; mitral valve; pannus; prosthesis failure; vascular endothelial growth factor.

Anahtar sözcükler: Aort kapağı; kalp kapak hastalığı; kalp kapak protezi; matriks metalloproteinaz; mitral kapak; pannus; protez başarısızlığı; vasküler endotelyal büyüme faktörü.