

Glandular Cardiac Myxoma with Associated Human Papilloma Virus Infection: Case Report

Human Papilloma Virus İlişkili Glandüler Kardiyak Miksoma

Canten TATAROĞLU,^a
Sevin KIRDAR,^b
Füruzan DÖĞER,^a
Nesibe ÇETİN,^a
Ceyhun CEYHAN,^c
Mehmet BOĞA^d

Departments of
^aMedical Pathology,
^bMedical Microbiology,
^cCardiology,
^dCardiovascular Surgery,
Adnan Menderes University
Faculty of Medicine, Aydın

Geliş Tarihi/Received: 03.10.2011
Kabul Tarihi/Accepted: 16.04.2012

Yazışma Adresi/Correspondence:
Canten TATAROĞLU
Adnan Menderes University
Faculty of Medicine,
Department of Medical Pathology, Aydın,
TÜRKİYE/TURKEY
cantent@yahoo.com

ABSTRACT Cardiac myxoma is the most common tumor of the heart, but glandular epithelial differentiation is a very rare phenomenon. A 71-year-old woman presented with a 6-month history of dyspnea on exertion. Echocardiogram revealed a mass arising from the interatrial septum and filling up the right atrium. Surgery was performed after a diagnosis of cardiac myxoma. Histological examination of the lesion demonstrated glandular differentiation. Paraffin embedded tissue samples were investigated for viral genomic DNA by PCR and a panel of immunohistochemistry. The panel confirmed this to a glandular variant of cardiac myxoma. HPV6 and HPV18 DNA were detected additionally. Here, we report a case of right atrial glandular myxoma, which was HPV positive.

Key Words: Myxoma; heart neoplasms; human papillomavirus 18; human papillomavirus 6

ÖZET Kardiyak miksoma kalpte en sık görülen tümördür fakat tümörde glandüler epiteliyal diferansiyasyon nadir bir bulgudur. 71 yaşında kadın hasta ekzersizle ortaya çıkan 6 aydır devam eden dispne şikayeti ile polikliniğimize başvurdu. Ekokardiyogramda interatriyal septumdan gelişen ve sağ atriyumunu dolduran kitle tespit edildi. Kardiyak miksoma ön tanısından sonra cerrahi eksizyon yapıldı. Histolojik bakıda lezyon glandüler farklılaşma sergilemekteydi. Parafine gömülü dokulardan PCR ile viral genomik DNA ve immünhistokimya çalışıldı. İmmünhistokimyasal panel ile kitle kardiyak miksomanın glandüler varyantı ile uyumlu bulundu. Ayrıca dokuda HPV 6 ve HPV 18 DNA'sı tespit edildi. Biz burada HPV pozitifliği gösteren glandüler kardiyak miksomalı bir olgu sunuyoruz.

Anahtar Kelimeler: Miksoma; kalp tümörleri; insan papillomavirüsü 18; insan papillomavirüsü 6

Türkiye Klinikleri J Cardiovasc Sci 2013;25(2):99-104

Primary neoplasms of the heart are rare tumors. Cardiac myxomas are the most common cardiac tumors in adults, and arise mostly in the left atrial wall. They make up about 50% of cardiac neoplasms. Myxomas are generally benign tumors, although exceptional malignant myxomas have been reported.¹⁻⁴ Clinic features of myxomas are systemic arterial embolism, obstruction of intracardiac blood flow, and constitutional signs such as fever, fatigue, weight loss and laboratory abnormalities.⁵ Glandular epithelial differentiation is very rare and found in only about 1% of cardiac myxomas, and the histogenesis of the glandular component is unclear.^{2,6,7} The origin of the glands has been attributed to epithelial differentiation of

a totipotent cardiomyogenic precursor, derivation from foregut remnants, bronchial or alveolar epithelium, mesothelium and germ cells or entrapped embryonal rests in the tumor.^{7,8}

Here we report an unusual form of myxoma, arising from the interatrial septum which had glandular structures and multinucleated giant cells, and additionally included Human Papilloma Virus (HPV) detected by PCR. To the best of our knowledge this is the first case of HPV infection associated with glandular atrial myxoma.

CASE REPORT

A 71 year old hypertensive diabetic woman presented with dyspnea on exertion of 6 month duration. She has been on chronic hemodialysis for renal failure. By physical examination the tricuspid valve insufficiency revealed systolic murmur. The woman had no HPV associated lesions anywhere by your body. Transthoracic echocardiography instead of echo of the chest revealed a mass arising from the interatrial septum and filling up the right atrium. Surgery was performed after a preliminary diagnosis of cardiac myxoma. A macroscopic examination showed that the tumor was 5x3x1.5 cm at its largest diameter. Macroscopically the tumor was friable and the cut surface was gelatinous with focal hemorrhagic regions. Hematoxylin-Eosin stained sections of paraffin embedded material showed polyhedral, stellate or spindle shaped cells which were lying in a myxoid background. The cells had oval nuclei and a moderate amount of eosinophilic cytoplasm. The tumor cells were arranged as single cells, short cords or vasoformative ring structures. Mitosis was absent. The myxoid background included focal hemorrhage, hemosiderin-laden macrophages, lymphocytes, plasma cells and neutrophils (Figure 1). A peculiar form of fibrosis (gamna bodies) was present (Figure 2). Some sections studied showed multinucleated giant cells (Figure 3). Glandular elements were irregularly shaped tubular structures lined by a single layer of cuboidal to columnar cells with goblet like cells. The nuclei of the glandular cells were larger and more vesiculated than those of typical myxoma cells (Figure 4).

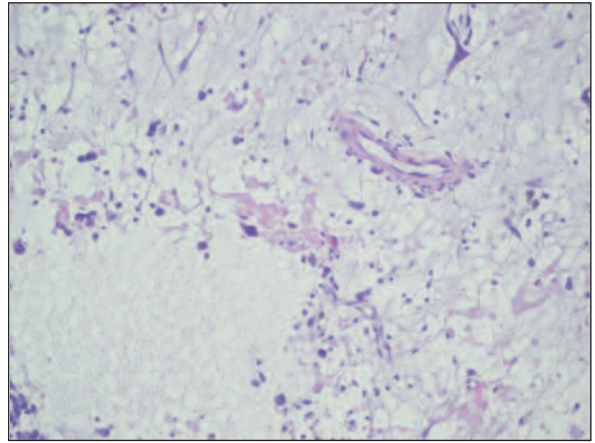


FIGURE 1: Stellat and spindle shaped cells in a myxoid stroma (HE, x400).
(See color figure at <http://cardiovascular.turkiyeklinikleri.com/>)

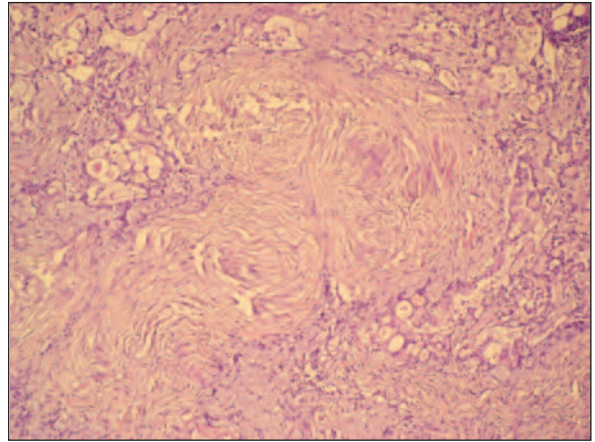


FIGURE 2: Gamna body in the stroma (HE, x200).
(See color figure at <http://cardiovascular.turkiyeklinikleri.com/>)

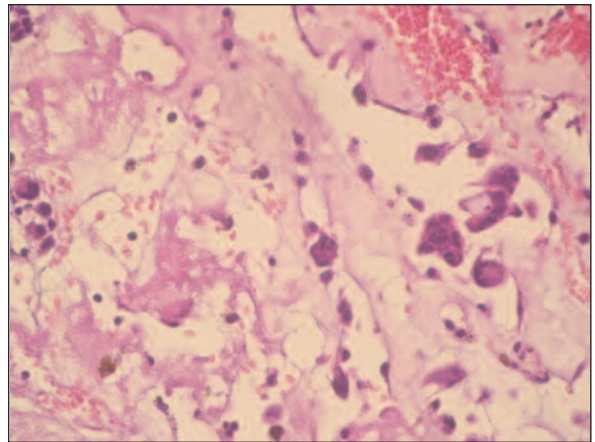


FIGURE 3: Multinucleated giant cells (HE, x400).
(See color figure at <http://cardiovascular.turkiyeklinikleri.com/>)

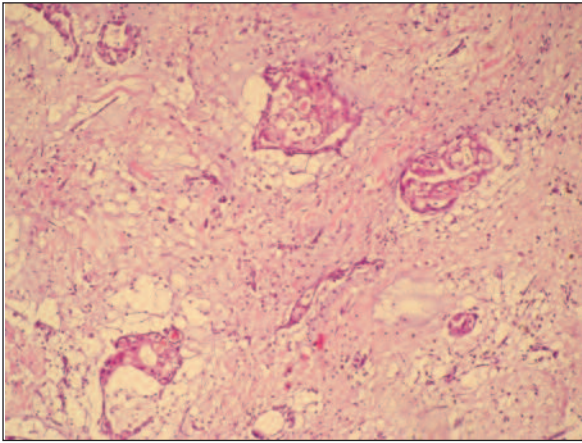


FIGURE 4: The glandular component of myxoma (HE, x100).
(See color figure at <http://cardiovascular.turkiyeklinikleri.com/>)

HISTOCHEMISTRY

The glandular cells were mucin secreting, as confirmed by positive Alcian Blue and mucicarmine staining.

IMMUNOHISTOCHEMISTRY

The formalin fixed, paraffin embedded sections were cut into 4 μ m sections. These sections were deparaffinized with xylene and hydrated through graded alcohols into water. Staining was performed by the avidine-biotin immunoperoxidase method for EMA, CK7, CK20, CEA, CD34, calretinin, Actin and Desmin. Finally the slides were treated with DAB substrate system (Labvision, Fremont, CA, USA). The sections were rinsed in water and were counterstained with Mayer's hematoxyline. The glandular cells were positive for EMA, CK7, calretinin, cyclin-D1, MUC1, MUC5 and CEA (Figure 5). CEA staining was in the luminal edge of the cells. CK7 and EMA staining were cytoplasmic. In the glandular cells and spindle shaped stromal cells were positive cytoplasmic staining with panHPV. Only in two or three cells were nuclear staining. There was no expression for CK20, S100, MUC2, MUC6, MOC31, Kromogranin, TTF-1, Tag72, CD30, EBV, Actin or Desmin. In the vasoformative structures only the innermost cells lining the lumen were stained with CD34 antibody (Figure 6). The ki-67 proliferating index was very low (less than 1%). The list of primary antibodies used is showed in table (Table 1).

PCR ANALYSIS

DNA was extracted from the tissue samples using a NucleoSpin Tissue Kit (Macherey & Nagel, Düren, Germany) in accordance with the manufacturer's instructions. We used a direct polymerase chain reaction (PCR) method for detecting HPV. Five μ L of DNA extract was used for PCR. To detect a broad range of HPV genotypes simultaneously, the consensus primers MY09 and MY11 were used. Primers are targeted to a conserved region of the L1 gene found in all HPV subtypes and amplify a fragment of 450 base pairs. Amplifications were carried out in a Mastercycler (Eppendorf, Germany) denaturation at 94°C for 45 seconds, primer

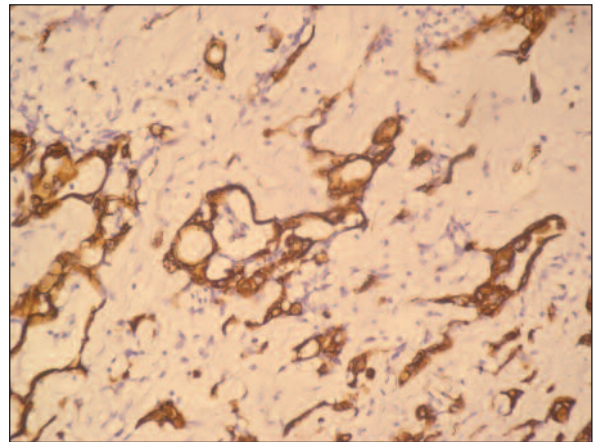


FIGURE 5: Immunohistochemical stain for cytokeratin 7 showing diffuse positive in the epithelial cells (CK7, x200).
(See color figure at <http://cardiovascular.turkiyeklinikleri.com/>)

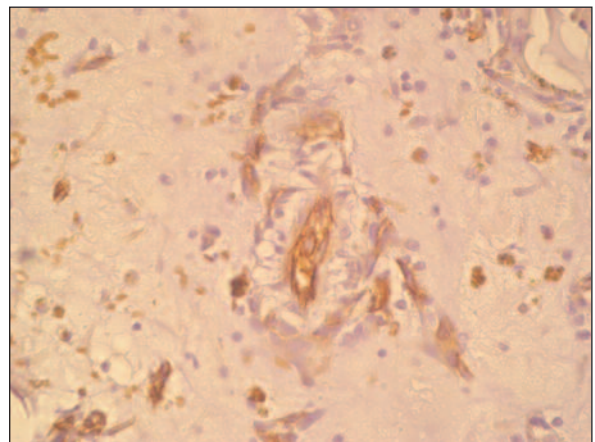
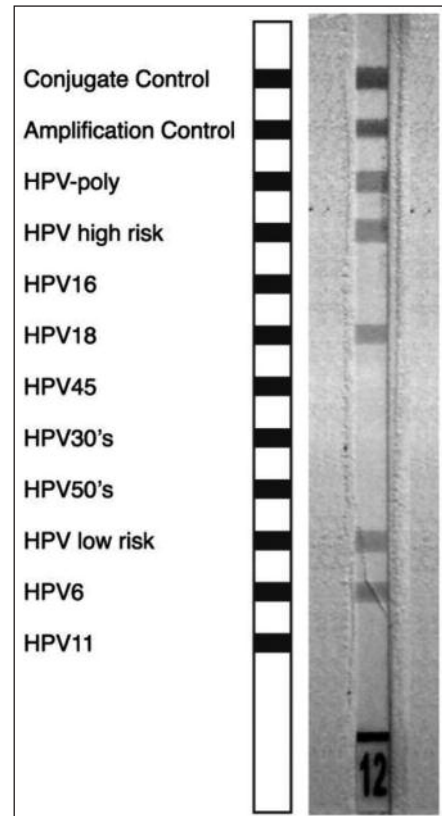


FIGURE 6: In the vasoformative structures only the innermost cells lining the lumen were stained with CD34 antibody (CD34, x200).
(See color figure at <http://cardiovascular.turkiyeklinikleri.com/>)

TABLE 1: List of primary antibodies used.

Marker	Species	Clone	Source	Expression in glandular cells
CD34	Mouse	RT4-END	Novocastra	-
EMA	Mouse	E29	Scy Tek	+
CK7	Rabbit	E12351	SpringBioscience	+
CK20	Rabbit	E16444	SpringBioscience	-
CEA	Rabbit	E2971	SpringBioscience	Luminal +
MUC1	Rabbit	RB-9222-R7	Neomarkers	+
MUC5	Mouse	MS-145-R7	Neomarkers	+
Calretinin	Mouse	Z11-E3	Zymed	+
MUC2	Mouse	MS-1037-R7	ThermoScientific	-
MUC6	Mouse	CLHS	Neomarkers	-
MOC31	Mouse	MS-825-R7	ThermoScientific	-
Kromogranin	Rabbit	SP12	SpringBioscience	-
TTF-1	Mouse	867G3/1	Zymed	-
Tag72	Mouse	HB-STn1	Dako	-
CD30	Mouse	RT4-CD30	Novocastra	-
EBV	Mouse	C51-CS4	DiagnosticBiosystems	-
Aktin	Mouse	E14341	SpringBioscience	-
Desmin	Rabbit	E257R40	SpringBioscience	-
HPV Ab-3	Mouse	MS-1826-R7	Thermo-Scientific	Cytoplasmic

annealing at 55°C for 45 seconds, DNA extension at 72°C for 1 minute, and lastly DNA extension at 72°C for 7 minutes. A total of 35 cycles were used for the amplification. Amplicons electrophoresis was done on 2% agarose gel. Clinical samples were checked for DNA quality and the absence of inhibitors of amplification by analysis for the human β -globin gene.⁹ The HPV line assay kit (GenID GmbH, Straßberg, Germany) was used for HPV DNA amplification and genotyping with reverse dot blot hybridization by following the manufacturer's protocol. Briefly, 5 μ l of each sample was amplified with biotinylated primers, which amplify the L1 open reading frame. Thereafter, the biotin-label amplified DNA was hybridized with sequence-specific oligonucleotide probes (SSOP) for HPV high-risk types 16, 18, 45, group detection of thirties and fifties high risk types (HPV types 31, 33, 35, 39 and 51, 52, 53), and the low-risk types 6 and 11. HPV DNA was determined in the paraffin embedded sections. We found both HPV type 18 as a high risk type and type 6 as a low risk type in the HPV positive sample (Figure 7). Epstein-Barr virus (EBV) and Herpes simplex virus type 1 and 2

**FIGURE 7:** HPV genotyping using Line Probe Assay. Lane 1: assay control bands Lane 2: HPV 18 and HPV6.

were also tested for in the paraffin embedded sections, but results for the two viruses were negative.

DISCUSSION

Cardiac myxoma is reported to occur in about 50% of primary cardiac tumors. The clinical presentation of myxoma depends upon its location and size. It manifests with a variety of symptoms, including congestive heart failure, systemic embolic events, palpitations, syncope and heart murmurs.^{10,11} Shimamoto et al. suggested that clinical presentation correlated with gross tumor features. Solid tumors were likely with symptoms of congestive heart failure and papillary tumors were likely with symptoms of systemic embolic events.¹² Histological characteristics in the diagnosis of classical myxoma are spindle shaped cells lying in a myxoid background. Gamna-gandy bodies, multinucleated giant cells, hemosiderin laden macrophages, vasoformative ring structures, inflammatory cells in the background are the other histopathological findings. In addition to these features our patient demonstrated glandular differentiation and HPV positivity. This study is the first report of a glandular cardiac myxoma including HPV6 and HPV18 viral DNA. It is however not clear whether HPV infection results in the development of myxoma or whether the virus infects the susceptible cells in myxomas. Li et al. hypothesized that HSV-1 may be involved in the pathogenesis of cardiac myxoma.¹³ In another study the authors suggest that some characteristics imply that HPV and EBV may play a role in cardiac myxoma; however, HPV or EBV was not detected in any of the patient samples in the study.¹⁴

Glandular differentiation is a very rare feature, and constitutes 2% of all cardiac myxomas. Recog-

nition of the glands is important as they can mimic metastatic adenocarcinoma.¹⁵ The histological distinction of metastatic carcinoma from glandular myxoma is the demonstration of areas of diagnostically typical myxoma within the tumor, and the atypical glands including abnormal mitosis and necrosis. The glandular components stained positively for epithelial markers. CK7, EMA, MUC1, MUC5 and calretinin were diffuse cytoplasmic positive and CEA was positive at the luminal edge of the cells, but the other epithelial markers such as CK20, MUC2, MUC6 and MOC31 were negative. These findings suggest that the glands in cardiac myxomas may recapitulate foregut derivatives, such as the upper gastrointestinal glands.⁸ Chu et al. examined the immunohistochemical expression of MUC1, MUC2 and MUC5AC in cardiac myxomas.^{16,17} Our results paralleled with these findings except MUC2. Notably, the immunohistochemical expressions of mucins in myxomas should be further evaluated. Additionally Kromogranin, TTF-1, Tag72, CD30, Actin, Desmin, and EBV were immunohistochemically negative. The histochemical and immunohistochemical panel confirmed this to be a glandular variant of cardiac myxoma. Detailed immunohistochemical analysis of mucins has been performed in this glandular myxoma. Moreover we found HPV6 and HPV18 DNA in paraffin embedded tissues.

It is concluded that there is an association between glandular myxoma and HPV. However, this association is not enough in the explanation of etiology of cardiac myxoma. It is recommended that HPV as an etiological factor should be evaluated in further studies in cardiac myxomas.

REFERENCES

1. Reyhanoğlu H, Kesici M, Ertürk M, Durmaz İ, Ertugrul G, Narin C. [Resecting left atrial wall together with myxoma in case of myxoma invasion into left atrial wall: surgical technique]. *Turkiye Klinikleri J Cardiovasc Sci* 2009;21(2):297-300.
2. Eckhardt BP, Dommann-Scherrer CC, Stuckmann G, Zollkofer CL, Wentz KU. Giant cardiac myxoma with malignant transformed glandular structures. *Eur Radiol* 2003;13(9):2099-102.
3. Curschellas E, Toia D, Borner M, Mihatsch MJ, Gudat F. Cardiac myxomas: immunohistochemical study of benign and malignant variants. *Virchows Arch A Pathol Anat Histopathol* 1991;418(6):485-91.
4. Moiyadi AV, Moiyadi AA, Sampath S, Kalpana SR, Mahadevan A, Shankar SK, et al. Intracranial metastasis from a glandular variant of atrial myxoma. *Acta Neurochir (Wien)* 2007;149(11):1157-62.
5. Endo A, Ohtahara A, Kinugawa T, Ogino K, Hisatome I, Shigemasa C. Characteristics of cardiac myxoma with constitutional signs: a multi-center study in Japan. *Clin Cardiol* 2002;25(8):367-70.

6. Namura O, Saitoh M, Moro H, Watanabe H, Sogawa M, Nishikura K, et al. A case of biatrial multiple myxomas with glandular structure. *Ann Thorac Cardiovasc Surg* 2007;13(6):423-7.
7. den Bakker MA, Dinjens WN, Bekkers JA. Cardiac myxoma with atypical glandular component, report of a case. *Histopathology* 2006;48(2):206-8.
8. Pucci A, Bartoloni G, Tessitore E, Carney JA, Papotti M. Cytokeratin profile and neuroendocrine cells in the glandular component of cardiac myxoma. *Virchows Arch* 2003;443(5): 618-24.
9. Giovannelli L, Campisi G, Lama A, Giambalvo O, Osborn J, Margiotta V, et al. Human papillomavirus DNA in oral mucosal lesions. *J Infect Dis* 2002;185(6):833-6.
10. Swartz MF, Lutz CJ, Chandan VS, Landas S, Fink GW. Atrial myxomas: pathologic types, tumor location, and presenting symptoms. *J Card Surg* 2006;21(4):435-40.
11. Liu Y, Xu ML. Cardiac myxoma with glandular component: case report. *Chin Med J (Engl)* 2006;119(2):174-6.
12. Shimono T, Makino S, Kanamori Y, Kinoshita T, Yada I. Left atrial myxomas. Using gross anatomic tumor types to determine clinical features and coronary angiographic findings. *Chest* 1995;107(3):674-9.
13. Li Y, Pan Z, Ji Y, Sheppard M, Jeffries DJ, Archard LC, et al. Herpes simplex virus type 1 infection associated with atrial myxoma. *Am J Pathol* 2003;163(6):2407-12.
14. Chu PH, Jung SM, Lin HC, Yeh CH, Wu HH, Shiu TF, et al. Caspase-3-dependent apoptosis in cardiac myxoma: not associated with human papillomavirus or Epstein-Barr virus. *Mod Pathol* 2005;18(6):822-7.
15. Travis WD, Brambilla E, Hermelink HKM, Harris CC. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart: Benign tumours of pluripotent mesenchyme. In: Travis WD, ed. *World Health Organization Classification of Tumors*. Lyon: IARC Press; 2004. p.260-4.
16. Chu PH, Jung SM, Yeh TS, Lin HC, Chu JJ. MUC1, MUC2 and MUC5AC expressions in cardiac myxoma. *Virchows Arch* 2005;446(1): 52-5.
17. Chu PH, Jung SM, Yeh CH, Yeh TS, Wang CL. Mucin gene expression in cardiac myxoma. *Int J Clin Pract* 2004;58(3):306-9.