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Research Article

Detection of Herplex Simplex Virus-1 and -2 in Cardiac Myxomas

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The etiology of sporadic cardiac myxomas remains elusive. The tendency for these lesions to recur following resection, their immunopathological characteristics, along with their histological and molecular profile, may implicate the presence of an infective agent in this type of tumor. In this study, we investigated the presence of herpes simplex virus (HSV) DNA in a cohort of cardiac myxomas in a tertiary referral centre. Twenty-nine formalin-fixed paraffin-embedded (FFPE) sporadic cardiac myxomas were obtained, 17 of which were shown to be informative. These were compared to 19 macroscopically and microscopically normal heart tissue specimens. The detection of HSV-1 and -2 genomic sequences was achieved with the use of a combined nested PCR-Restriction Fragment Length Polymorphism methodology. The presence of HSV-1 and/or -2 DNA was demonstrated in 6 of 17 (35%) informative sporadic cardiac myxomas, whereas no HSV DNA was detected in normal heart tissues ($P < 0.01$). The existence of HSV-1/2 DNA in sporadic cardiac myxomas, along with its absence from normal heart tissues, reinforces the possibility that HSV infection might be involved in the development of these lesions. Our findings raise the point of anti-HSV medication postsurgically with a potential benefit in reducing the rate of recurrences.

1. Introduction

Primary heart neoplasms are rare and encountered in approximately 0.056–0.02% of autopsy series. These affect patients of all ages, with the vast majority being benign (75–80%), and with the females more often affected than males. Myxoma is the commonest primary cardiac neoplasm with a malignant potential, accounting for approximately 75% of all cases [1], having an annual incidence of about $1/10^6$ and a recurrence rate of 2-3% [2, 3]. Some cases are familial and appear to have an autosomal dominant transmission [4]. These are frequently single, affecting mainly the atrial fossa ovalis in the left atrium [1] as part of the Carney complex, which has been found to be associated with the germline mutation *PRKAR1A* encoding Protein Kinase A Regulatory subunit type 1A. Only familiar forms of myxomas have been associated with the mutation; sporadic cases of myxomas have no association with it [4, 5]. Sporadic myxomas, represent the majority of the diagnosed myxomas and their etiology remains elusive. Although recurrences of myxomas have mostly been attributed to incomplete excision, this explanation does not account for recurrences at distant sites within the atria [6].

Various indications point towards the involvement of Herpes Simplex Viruses (HSVs) in sporadic cardiac myxoma pathogenesis. The endocardium of the atrial septum, where the atrial myxoma mainly originates from, is rich in sensory nerves. In turn, myxoma cells appear to be derived from endocardial sensory nerve tissue [7]. The life cycle of HSV is characterized by latency in sensory or autonomic ganglia

that can be maintained for life in the host. Periodically, the virus can be reactivated by stimuli causing either viral shedding or recurrent infection of the affected nerve [8]. In addition, a report in Chinese patients has suggested HSV-1 presence in sporadic atrial myxomas [9]. Our aim was to examine whether HSV-1, 2 DNA could be detected in a cohort of patients with cardiac myxomas more frequently than in normal control hearts.

2. Materials and Methods

Twenty-nine tissue specimens originating from patients with typical clinicopathological features of sporadic cardiac myxoma were examined. The tissue specimens were obtained from Onassis Cardiac Surgery Centre (1996–2003) from patients admitted for surgical removal of the lesion. Tissue specimens from 19 macroscopically and microscopically normal hearts, provided by the forensic department of the university, served as controls. Specific care was taken so as the material from the normal controls was taken from the atria. The control specimens were matched for age and sex with the myxoma specimens. All tissues were formalin-fixed, paraffin-embedded. Use of the myxoma material followed written permission by the subjects, while the local Ethics Committee approved further experimentation. The study was performed according to the requirements of the revised (1983) Helsinki Declaration of 1975. Whole blood from previously tested HSV-1/2-infected patients was used as positive control. Histopathological evaluation was performed by hematoxylin and eosin staining.

DNA extraction was performed according to the conventional phenol/chloroform protocol with slight modifications [10]. The *IFN-γ* house-keeping gene was used to assess the integrity and yield of the extracted DNA [11].

The primers used in the first-round amplification reaction are the following: 5′-TGCTCCTACAACAAGTC-3′ and 5′-CGGTGCTCCAGGATAAA-3′ with annealing temperature 55°C and respective product size 200 bp. In the second-round amplification reaction the following primers were employed: 5′-ATCCGAACGCAGCCCCGCTG-3′ and 5′-TCTCCGTCCAGTCGTTTATCTTC-3′ (reverse) with annealing temperature 60°C and corresponding product size 142 bp. They are based on those reported for the conserved HSV *glycoprotein D* gene ((GenBank accession numbers: X14112 (HSV-1), Z86099 (HSV-2)) [12]. PCR reactions were performed as previously described [11]. The primers for *Interferon γ* (*IFNγ*) are the following: 5′-CTCTTTTCTTTCCCGATAGGT-3′ and 5′-CTGGGATGCTCTTCGACCTCG-3′ with annealing temperature 57°C and respective product size 151 bp.

Restriction Fragment Length Polymorphism (RFLP) analysis was performed on HSV-positive nested-PCR products, digested with the restriction enzyme M*sp*I (New England Biolabs) at 37°C for 4 h. RFLP digests were size-fractionated by electrophoresis on 7.5%-polyacrylamide gel, stained with ethidium bromide and photographed under UV light.

Data are expressed as mean ± 1 standard deviation (S.D.) for continuous variables and as frequency (percentage %) for categorical data. The normality of the distributions was assessed with Kolmogorov-Smirnov test and graphical methods. Comparisons of continuous variables were performed using Mann-Whitney's *U* nonparametric test. Categorical data were compared by Fisher's exact test. The necessary number of control samples was determined by power analysis (G*Power 3, Universität Kiel, Germany). Differences were considered to be statistically significant if the null hypothesis could be rejected with >95% confidence ($P < 0.05$).

Table 1: Characteristics of the myxoma patients (all and informative cases) and the normal controls; no statistical significance was observed in baseline characteristics between groups.

Characteristics	Myxoma patients $N = 29$	Normal controls $N = 19$	Informative myxomas $N = 17$
Age (mean ± SD) years	58 ± 13	61 ± 18	57 ± 14
Female *n* (%)	25 (86.2)	16 (84.2)	14 (82.4)

3. Results

The demographic characteristics of the myxoma patients (all and informative cases) and the normal control hearts are depicted in Table 1. Twenty patients presented with various clinical manifestation (strokes, pulmonary embolization), while for the remaining 9 patients the myxoma was an incidental finding on cardiac ultrasound. The myxoma was excised from the right atrium in 22 patients (75.9%) from the left atrium in 2 patients (6.9%), while in 5 (17.2%) patients the lesion was excised from the right ventricle. Histological evaluation clearly exhibited the characteristic gel-like stroma of mucopolysaccharides on hematoxylin-eosin (H&E) staining (Figure 1(a)) [13]. In this cohort of specimens, 17/29 cases produced appropriate DNA yields for further HSV detection. A clear 142-bp band corresponding to both HSV-1 and HSV-2 genomes was obtained following PCR amplification in 6 myxoma cases, accounting for 35.3% of the sample (Figure 1(b)). In contrast, HSV DNA was absent from the 19 normal heart tissues tested ($P < 0.01$) (Figure 2). Five of the infected cases originated from female patients from the left atrial cavity, and one specimen was removed from the right atrial chamber of a male patient. RLFP analysis allowed the identification of the infectious viral type in 4/6 HSV-positive cases (Figure 2). In fact, two atrial myxomas were found to harbor HSV-1, one was infected by HSV-2, while both viruses were present in an additional case (Figure 3).

4. Discussion

This study has shown that HSV DNA is detected significantly more frequently in cardiac myxomas than in their normal counterparts. The demonstration of HSV-1 infection lies in accordance with a study performed on the Chinese population, which reported on the occurrence of the virus in a subset of sporadic cardiac myxomas [9]. The authors found that the examination of the viral DNA is more sensitive than

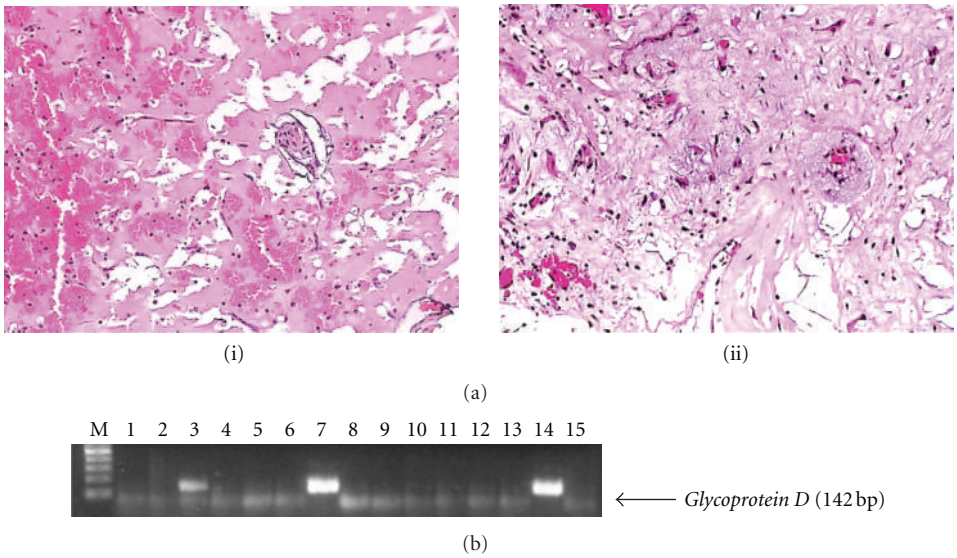


Figure 1: (a) Histological evaluation of representative HSV-positive and HSV-negative cardiac myxoma cases. Hematoxylin-eosin (H&E) staining of myxoma tissue sections revealed sparsely cellular lesions with a characteristic gel-like stroma of acid mucopolysaccharides, containing individual small cells with sparse cytoplasms forming stellate protrusions into the stroma. (i) HSV-negative left atrial myxoma sample (case 1). (ii) HSV-positive right ventricle myxoma sample (case 7) (×200 magnification, H&E staining). (b) Detection of HSV DNA in representative atrial myxoma specimens. M: 100 bp molecular marker (New England Biolabs), 1–13: atrial myxoma samples, cases 1, 2, 4–6, 8–13: HSV-negative samples, cases 3, 7: HSV-positive samples, 14: positive control (blood sample), and 15: negative control (normal heart tissue).

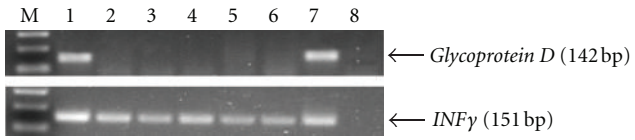


Figure 2: Absence of HSV DNA from normal heart tissues. HSV and *IFN-γ* PCR in representative cases with normal heart tissues. PCR. M: 100 bp molecular marker (New England Biolabs), 1 and 7: positive control (blood sample), 2–6: normal heart tissues, and 8: negative control (distilled water). *IFN-γ* was used to assess the quality and quantity of the examined DNA.

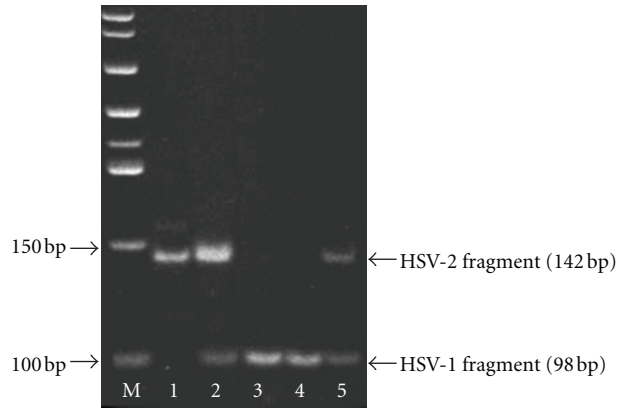
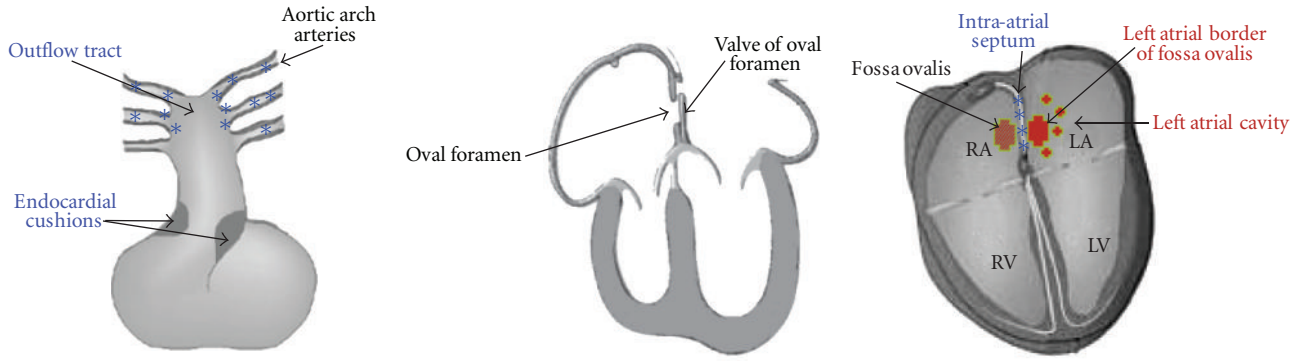


Figure 3: HSV typing via RFLP analysis. Electrophoresis of representative nested-PCR products, positive for HSV, digested with M*sp*I. M: 50 bp marker (New England Biolabs), 1: undigested nested-PCR product (142 bp), 2: positive control containing both HSV-1 (98 bp) and HSV-2 (142 bp) genomic sequences, 3-4: cases infected by HSV-1, and 5: case harboring both HSV-1 and -2.

the immunohistochemical evaluation of HSV-1 in cardiac myxomas, which may provide an explanation for the findings of a recent study showing no association between HSV and cardiac myxomas [14]. The present study was adequately powered to detect statistical differences between cardiac myxomas and normal hearts regarding the presence of HSV. In addition, the detection of HSV-2 as the infectious agent in two myxoma cases reflects a novel finding. Two others members of Herpesviridae family have been long ago established as oncogenic agents, Epstein-Barr virus (EBV) and Human Herpes Virus type 8 (HHV-8) [15]. EBV is associated with the development of Burkitt's lymphoma, Hodgkin's lymphoma (B-cell), and nasopharyngeal carcinoma, and HHV-8 is having a causal role for the development of sarcoma Kaposi.

Common clinical manifestations of myxomas are strokes, peripheral or pulmonary embolization, fever, weight loss, high sedimentation rate, anemia, and leucocytosis [1]. At an immunological level, a CD8+ cellular infiltrate and elevated IL-6 levels have been observed [9, 16, 17]. Additionally, the molecular profile commonly associated with neoplastic disease, such as mutations in gatekeeper genes, is absent [18]. It is likely that, in certain susceptible individuals, the development of cardiac myxoma is the result of an intense inflammatory process, secondary to an inciting agent. In turn persistent inflammation increases DNA mutations and overall genomic instability favoring



(a) Embryonic heart (33 days) (b) Newborn heart (c) Adult heart

FIGURE 4: Neural crest embryological remnants in the adult heart and localization of cardiac myxomas. (a) Embryonic heart. Blue stars indicate the localization of neural crest cells from the pharyngeal mesoderm, which migrate through the aortic arch arteries to the outflow tract and the endocardial cushions. (b) Newborn heart. The Fossa ovalis in the adult heart is an embryonic remnant of the foramen ovale, which closes shortly after birth. (c) Adult heart. Red crosses indicate the attachment of HSV particles to cardiac myxomas. Blue stars indicate autonomic nerve fibers. RA: right atrium, RV: right ventricle, LA: left atrium, and LV: left ventricle.

neoplastic transformation [19]. It has been well established that chronic inflammation through several inflammatory mediators including cytokines, reactive oxygen, and nitrogen species induce genetic instability, a hallmark of cancer [20]. Alternatively, and not mutually exclusively, the infectious agent may disrupt critical signaling pathways implicated in cell control. Concerning Herpesviridae family, both EBV and HHV-8 target crucial components of cellular machinery including NFκB and p53 as well as the Wnt pathway through the stabilization of b-catenin [15]. The latter is achieved by the production of certain viral proteins which abrogate the ubiquitination of b-catenin. Interestingly, HSV-1 encodes UL-36, a protein with deubiquitinating activity [21]. The examination of the effect of UL-36 in b-catenin stabilization remains an attractive issue that may shed light on the potential oncogenic properties of HSV-1. Altogether, the above may provide an explanation for the presence of chromosomal aberrations including aneuploidy in a subset of cardiac myxomas [22, 23].

Mucopolysaccharides found in cardiac myxomas have been implicated as receptors of HSV particles during infection [24]. The fact that 6 cardiac myxoma specimens were found to be HSV positive in contrast to the complete absence of HSV in the control group despite the reported prevalence of these viruses in the general population [25] renders the possibility of endocardial infection to be a random event rather unlikely.

The role of HSVs in the pathogenesis of cardiac myxoma is still unclear. Whether the viruses are among the inciting agents for the development of the lesion or simply represent opportunistic pathogens with a tropism for myxomatous endocardium remains to be answered [9]. One may assume that cardiac myxomas arise as a direct consequence of lytic-HSV infection. The viral life cycle is characterized by latency in sensory or autonomic ganglia. Periodically, the virus can be activated causing viral shedding or recurrent infection [8]. The majority of myxomas occur on the left atrial cavity, and the most common site of attachment is the left atrial border of the fossa ovalis [1]. It is well established that the endocardium of the atrial septum is rich in autonomic nerve fibers and, thus, may serve as a portal for the entry of HSVs. Experimentally induced HSV carditis in mice suggests that HSV-1 is able to affect the endocardium via these nerve cells [26]. Furthermore, studies on sporadic atrial myxomas have shown that these lesions express antigens, which are representative of a neural cell component, such as neurone-specific enolase, S100, synaptophysin-38, CD34, alpha-smooth muscle actin, and calretinin [7, 13]. Of note, IL-6, detectable at high levels in myxomas, has been associated with reactivation of latent HSV [16, 17]. Moreover, during embryonic development, noncardiac cells, particularly neural crest cells from the pharyngeal mesoderm, migrate through the outflow tract to the endocardial cushions [27, 28] (Figures 4(a) and 4(b)). The existence of cardiac neural crest cells is of nodal importance, since their absence results in congenital malformations [27]. However, their fate following heart development is poorly defined [29]. It is possible that HSV infects these embryological remnants, hence causing an intense inflammatory reaction, leading to myxoma development. These observations regarding the embryological origins of endocardial tissue may account both for HSV tropism towards this site and for the frequent recurrence of cardiac myxomas (Figure 4(c)).

Of particular interest is the recognition of HSV-2 as a potential cardiovascular pathogen. The virus has been implicated in coronary artery disease and carotid atherosclerosis [30]. Moreover, infection by HSV-2 seems to convey an increased risk for cardiovascular death and myocardial infarction [31].

This study has a number of limitations. Its major limitation is the small sample size. However, cardiac myxomas are extremely rare and the investigators powered the study enough to detect significant differences, if present. In addition, the cardiac samples that served as controls were received from apparently healthy hearts. Even if the investigators made sure that no cardiac involvement had

led to the death of the subjects and the hearts were found to be microscopically healthy, the possibility of a possible underlying cardiac disease cannot be excluded.

Since myxomas are clinically significant lesions, with potential life-threatening sequelae that affect patients of all ages and the only available treatment is complete resection of the tumor, the identification of possible underlying treatable causes is extremely important. Our work supports the occurrence of HSV in a subset of sporadic cardiac myxomas. While further studies are needed to clarify the role of HSV1 in cardiac myxoma pathogenesis, it is conceivable to trial suppressive anti-HSV drugs, such as acyclovir, after surgery in the HSV-positive patients, in order to avoid a possible recurrence of sporadic cardiac myxomas.

Authors' Contribution

I. S. Pateras and K. Evangelou contributed equally to this paper.

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