Journal of Clinical Virology 49 (2010) 290–295

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Polymyositis/dermatomyositis and nasopharyngeal carcinoma: The Epstein–Barr virus connection?

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ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form 12 July 2010
Accepted 28 August 2010

Keywords:
Epstein–Barr virus (EBV)
EBV nuclear antigen-1 (EBNA-1)
EBV DNA load
Polymyositis/dermatomyositis (PM/DM)
Nasopharyngeal carcinoma (NPC)

ABSTRACT

Background: Polymyositis (PM) and dermatomyositis (DM) are associated with high risk of nasopharyngeal carcinoma (NPC) in Asian countries. Epstein–Barr virus (EBV) might induce autoimmunity and malignancies in susceptible individuals.
Objectives: To investigate the association of EBV with PM/DM and NPC in PM/DM patients.
Study design: Serum levels of anti-EBV viral capsid antigens (VCA) and anti-EBV-coded nuclear antigens-1 (EBNA-1) antibodies were measured by ELISA, and EBV DNA loads were determined using real-time PCR for 98 PM/DM patients, 94 systemic lupus erythematosus (SLE) patients and 370 healthy controls (HC). Anti-transfer-RNA synthetase antibodies (ASA) were determined by radioimmunoprecipitation for PM/DM patients.
Results: Thirteen (13.3%) of PM/DM patients vs. none of SLE patients had detectable NPC. ASA were detectable in 31.7% of PM/DM without malignancy, while lack of ASA in any PM/DM patient with NPC. IgA anti-EBNA-1 were detectable in 30.6% of PM/DM patients and 31.9% of SLE patients, but only in 4.1% of HC (odds ratio [OR] 10.44 and 11.12 respectively, both $p < 0.001$). Significantly higher positivity for IgA anti-EBNA-1 were observed in PM/DM with NPC than in those without malignancy (OR 44.7, $p < 0.01$). Significantly higher positivity for EBV genome were observed in PM/DM with NPC than in those without malignancy (OR 43.9, $p < 0.01$), in SLE patients (OR 13.2, $p < 0.05$) and in HC (OR 99.4, $p < 0.001$). EBV DNA loads were significantly higher in PM/DM with NPC compared with those without malignancy and HC.
Conclusions: Our results showed a positive association of EBV with PM/DM and NPC. PM/DM patients who have IgA anti-EBNA-1 or increased EBV DNA loads should be highly suspected to have occult NPC.

1. Background

Polymyositis (PM)/dermatomyositis (DM) are inflammatory muscular diseases,[1,2] and are the outcome of autoimmunity that results in injuries to muscle fibers.[3–5] The best defined myositis-specific autoantigens are transfer RNA-synthase, and the prevalence of anti-synthetase antibodies (ASA) is up to 20–37% in PM/DM patients.[6,7] However, the etiopathogenesis of PM/DM remains unclear.

Viral infection might induce autoimmunity in susceptible individuals.[8,9] The reported seasonal occurrence of PM/DM indicates that viral infection could trigger this rheumatic disease.[10,11] Epstein–Barr virus (EBV) is a ubiquitous human gamma-herpesvirus with cell growth transforming ability that predominantly infects B-lymphocytes and elicits strong immune responses.[12] It has been suggested that rheumatic diseases arise as a consequence of antigenic cross-reactivity of anti-EBV antibodies.[13–15]

PM/DM is associated with a higher risk of malignancies.[16–18] Nasopharyngeal carcinoma (NPC) is the most common malignancy in Chinese PM/DM patients.[19–21] NPC has been proved to be an EBV-associated cancer.[22,23] Expression of EBV latent proteins and the association between NPC and EBV has been exploited to develop serologic markers and EBV DNA for this cancer.[24] In an EBV-associated disease study, IgA anti-EBNA-1 has been used as a

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doi:10.1016/j.jcv.2010.08.015

reliable screening test for NPC patients in Taiwan.[25] Recent studies showed that EBNA-1 was the only viral protein expressed in NPC, and C-terminal domain-derived epitopes could elicit CD4+ T-cell responses in muscle biopsies from DM patients.[26,4] Recently, serum EBV DNA is a molecular marker for NPC.[27] We hypothesized that EBV may play an important role in the pathogenesis of PM/DM, especially in those with NPC.

2. Objectives

This study is the first attempt to investigate the associations of EBV infection with clinical manifestations and the occurrence of NPC in PM/DM patients. We re-examine the association between EBV infection and PM/DM using serologic assays for IgA anti-EBNA-1 antibody responses to a fragment of EBNA-1 lacking cross-reactive epitopes, and using real-time quantitative polymerase chain reaction (RQ-PCR) to determine EBV DNA loads. To test whether the emergence of NPC in myositis patients was the consequence of immunosuppression, we enrolled patients with systemic lupus erythematosus (SLE) that is known to have clinical relevant EBV infection[28,29] as an additional control.

3. Study design

3.1. Patients and matched controls

Ninety-eight adult patients fulfilling the Bohan and Peter criteria of PM/DM[30,31] were enrolled. After investigation, all patients were treated with corticosteroids and immunosuppressive agents. Ninety-four consecutive patients fulfilling the 1997 revised criteria for SLE[32] and continuously receiving immunosuppressant were enrolled as disease control. Three hundred and seventy healthy volunteers who had no rheumatic disease or malignancy served as healthy controls (HC) by matching for sex and geographic location in Taiwan. The Ethics Committee of our Hospital approved this protocol and informed consent was obtained from each participant.

3.2. Determination of antinuclear antibodies (ANA) and ASA in PM/DM patients

ANA were screened against Hep-2 cells by indirect immunofluorescence using the FLUORHEPANA TEST kit (MBL, Japan). ASA were detected by RNA radioimmunoprecipitation (RIP) using the HeLa cell S^{100} extract as described previously.[33,34,7]

3.3. Determination of anti-EBV-VCA and anti-EBV-EBNA-1 antibodies

Serum levels of IgG anti-EBV-VCA were determined using ELISA according to manufacturer's instructions (IBL-Hamburg, Germany). Antibodies against the purified glutathione-S-transferase (GST)-EBNA-1 fusion protein containing the C-terminal domain of EBNA-1 residues 390–641 were measured by ELISA as previously reported[25] with slight modifications. For each assay, 3 positive controls (pools of 15 NPC sera) and 3 negative controls (pools of 18 normal sera) were included.

3.4. Determination of EBV DNA levels

DNA was extracted from sera using the High Pure Viral Nucleic Acid kit (Roche, Mannheim, Germany) according to manufacturer's instruction. EBV genome loads were determined by RQ-PCR assay and the primers and probes for detecting the *BamHI* W region of EBV were as reported.[27] The fluorogenic PCR was set up in a reaction volume of 50 μl using components supplied in a TaqMan PCR Core Reagent Kit (Applied Biosystems, USA). A calibration curve, using DNA from the EBV-positive Burkitt's lymphoma Raji cells as a standard, was run in parallel and in duplicate. Results were expressed as EBV copies/ml of serum. A parallel RQ-PCR analysis for the EBV EBNA-1 gene with similar conditions to that of *BamHI* W region was conducted to ensure the specificity of EBV genome, and exclude the possibility of the signals for mitochondrial DNA originated from erythrocytes that might contaminate the serum.

3.5. Statistical analysis

Chi-square test and Fisher's exact test were used to determine significant differences in categorical variables among groups. Data were expressed as odds ratio (OR) with exact 95% confidence interval (CI). The nonparametric Kruskal–Wallis test was used to determine the mean difference of copy numbers of EBV genome. *p*-Values less than 0.05 were considered statistically significant.

4. Results

4.1. Clinical characteristics and laboratory findings

Thirteen (13.3%) PM/DM patients had a detectable NPC, while none of SLE patients developed NPC during the 10-year follow-up period. As illustrated in Table 1, significantly lower proportion of females and interstitial lung disease was observed in PM/DM patients with NPC compared with those without malignancy. Interestingly, a lack of detectable ASA was observed in any myositis patient with NPC. There were no significant differences in the mean doses of daily corticosteroids or the proportion of used immunosuppressant between PM/DM patients and SLE patients, or between PM/DM patients with NPC and those without NPC.

All patients with NPC were dermatomyositis. Most patients (77%, 10/13) developed NPC within one year after diagnosis of myositis. Two patients developed NPC more than one year after the onset of myositis, while another patient presented with NPC before the onset of myositis (Table 2).

4.2. IgG anti-EBV-VCA, IgG and IgA anti-EBNA-1antibodies in PM/DM patients and controls

This study used anti-EBV antibodies titer ≥1:10 to define positive results. As illustrated in Table 3, significantly higher positive rates for IgA anti-EBNA-1 were observed in PM/DM patients and SLE patients than in HC (OR 10.44 and 11.12 respectively, both $p < 0.001$). Moreover, significantly higher positive rates for IgA anti-EBNA-1 were observed in PM/DM with NPC than in HC (OR 284, $p < 0.001$), in those without NPC (OR 44.7, $p < 0.01$), and in SLE patients (OR 25.6, $p < 0.01$, Table 4). However, there was no significant difference in the positive rate for IgG anti-EBV-VCA or IgG anti-EBNA-1 among these four groups.

4.3. EBV genome and DNA loads in PM/DM patients and controls

We used detectable (≥10 copies/ml of serum) circulating EBV DNA loads to define positive results. As illustrated in Table 3, significantly higher positive rates for EBV DNA were observed in PM/DM patients and SLE patients than in HC (OR 5.82 and 7.56 respectively, both $p < 0.001$). Moreover, significantly higher positive rates for circulating EBV genome were observed in PM/DM with NPC than in HC (OR 99.4, $p < 0.001$), in those without malignancy (OR 43.9, $p < 0.01$), and in SLE (OR 13.2, $p < 0.05$, Table 4).

Among individuals with detectable EBV genome, EBV DNA loads were significantly higher in PM/DM with NPC compared with PM/DM without malignancy and HC (mean ± SEM, 278,755.6 ± 187,431.5 copies/ml vs. 278.6 ± 68.9 copies/ml for