Prevalence of Mycosis Fungoides and Its Association with EBV and HTLV-1 in Pakistani Patients

Samina NOORALI, Nausheen YAQOOB, Muhammad Israr NASIR, Tariq MOATTER, Shahid PERVEZ

Department of Pathology, The Aga Khan University, Karachi, Pakistan

Mycosis fungoides (MF) is an indolent T cell lymphoma that is distinguished from other lymphomas by its initial appearance on the skin. The histologic diagnosis of MF may be difficult because there is significant overlap in the histologic features of neoplastic T cell infiltrates and inflammatory dermatoses. This T cell neoplasm commonly occurs in a mixed, reactive background and can show only a subtle degree of cytologic atypia, rendering histologic diagnosis difficult. In this study MF constituted 0.86% of all non-Hodgkin’s lymphoma (NHL) both T and B, as compared to the Western studies which have reported 0.5% prevalence for MF of all NHL. Polymerase chain reaction (PCR) technique was used to assess T cell clonality in paraffin-embedded skin biopsies clinically and pathologically suspicious for early MF. Out of the 14 cases diagnosed as MF, amplifiable DNA was isolated from 6 cases, which were further studied for T cell receptor (TcR) - β, γ, and δ chain gene rearrangements. Clonal product was seen in 4 out of 6 cases for β, γ, and δ TcR chain genes. Association for Epstein Barr virus (EBV) was observed in 3 out of 6 cases (50%) of MF. Although these 3 cases were positive for EBV by PCR, but were negative by in-situ hybridization (ISH). No heterogeneity was noted in these 3 cases of MF for BamHI E, K, N, and Z regions of EBV. All six cases were negative for HTLV-1 (tax region) by PCR. It was concluded that the prevalence of MF in Pakistani population is comparable to the Western data, and that EBV association to MF cases was higher than in Western studies. (Pathology Oncology Research Vol 8, No 3, 194-199)

Keywords: Mycosis fungoides (MF), EBV, HTLV-1, in situ hybridization (ISH), T cell receptor (TcR)

Introduction

Mycosis fungoides (MF) is a lymphoproliferative disorder involving neoplastic T lymphocytes. Infiltration of neoplastic cells is invariably demonstrated in the skin. Involvement of the lymph nodes, peripheral blood and viscer is also often found. The histological features of MF are characterised by marked epidermotropism of cytologically atypical T lymphocytes, with convoluted (cerebelliform) nuclear contours. These cells form a band like infiltrate in the upper papillary dermis and show formation of Pautrier’s microabscesses in the epidermis. There is variable admixture of histiocytes, eosinophils, and plasma cells. Abnormal cells must be present in the epidermis to make a definitive diagnosis of MF. It is estimated that in the USA, MF develops in 1,000 new patients per year and constitutes 0.5% of all NHLs. A clinical diagnosis of MF in its early stages can be difficult and even on histological examination at times is difficult to distinguish it from benign inflammatory dermatoses such as lymphomatoid drug reactions, contact dermatitis, lichen planus, spongotic dermatitides, pityriasis lichenoides et varioliformis acuta, follicular mucinosis etc, particularly when mixed cell populations with only occasional atypical cells are observed. MF may produce practically all of the patterns used for diagnosing inflammatory skin diseases. MF is characterized immunohistochemically by the presence of
(CD 3+) T cell expressing a T-helper/inducer cell immunophenotype (CD4+), with only rare cases having a T-suppressor cell immunophenotype (CD8+). Break points in chromosomes 1, 9, 14, and 17 and abnormalities involving the gene for the TcR α (14q11) have been noted frequently in patients with MF.

Kanavaros et al. and Peris et al. have shown no significant role for EBV in the development of cutaneous lymphoma. Whereas Dreno et al. have reported 32% prevalence of EBV in cutaneous T-cell lymphomas. Several studies have shown that nearly all patients with MF are HTLV-1 seronegative, but by using polymerase chain reaction (PCR) HTLV-1 DNA sequences has been detected in peripheral mononuclear cells and cutaneous lesions of some patients with MF. Other studies have shown no role for HTLV-1 in the development of MF.

The present study is the first to investigate the prevalence of MF, in a large tertiary care referral hospital receiving specimens from all over Pakistan, its association with EBV, HTLV-1 and genetic features to study T-cell receptor (TcR) gene rearrangements.

Materials and Methods
Histology and Immunohistochemistry

Specimens received in 10% neutral buffered formalin were processed under standardized conditions for paraffin embedding, cut at 5 μm thickness, and stained with hematoxylin and eosin (H&E). Immunohistochemical analysis was performed using CD45 (LCA), CD20 (L26), CD45RO (UCHL1) and CD3 antibodies (Dako, Denmark). Antibody binding was detected by Vectastain (Vector Laboratories Inc., USA) following incubation with specific antibodies. Finally, the slides were counterstained with Harris hematoxylin. Each assay included a negative and a positive control comprising tonsil tissues.

PCR Amplification of β-globin, EBV and HTLV-1 Genome

DNA from 14 MF patient biopsies, 14 reactive lymph nodes and 30 blood samples was extracted by Nucleon HT kit (Biosciences, USA), according to the manufacturer's instruction. Reactive lymph node biopsies and blood samples were used as control for the presence of EBV and HTLV-1. Purified DNA (500 ng) was added to a PCR mix containing 0.2 mM deoxynucleotide triphosphates (dNTPs), 1.5 mM MgCl2, 150 ng of each primer for β-globin and HTLV-1, 300 ng of each primer for EBV, 2.5 U Taq DNA polymerase (Advanced Biotechnologies, UK) in a total volume of 50 μl. After initial denaturation at 94°C for 5 mins, 45 cycles of denaturation at 94°C for 1 min, annealing 55°C (EBV and β-globin) or 58°C (HTLV-1) for 1.5 min and polymerization 72°C for 1.5 min were carried out in an automated thermal cycler (Perkin Elmer 9600, USA). The cycling program was ended with a final extension at 72°C for 7 minutes. PCR products of 240-bp for β-globin, 239-bp for EBV genome, and 159-bp for HTLV-1 were seen.

In Situ Hybridization (ISH)

Specimens positive for EBV by PCR were further processed for the cellular localization of EBV genome using in-situ hybridization (ISH) on fixed tissue sections. Subsequently, tissue sections were hybridized with either biotin labeled EBV, EBER-1 region specific probe or a positive control probe prepared in 20% dextran sulphate solution. A negative control slide was included with each run. The target RNA and probe were denatured at 100°C for 5 minutes and hybridized overnight. The hybridized probe was detected using ISH detection system supplied by Life Technologies, USA.

Determination of variation in isolated EBV strains by PCR-SSCP

Heterogeneity in EBV DNA was identified using PCR-SSCP as previously described by Dimitris et al. with slight modifications. The BamHI E, N, K and Z regions of EBV were examined for sequence variation. Primers, thermal cycling and product analysis were as reported previously. Briefly 5 μl of PCR product was mixed with 5 μl of gel loading dye (95% formamide, 10 mM NaOH, 0.05% bromophenol blue and 0.05% xylene cyanol) denatured at 95°C for 5 min, cooled on ice and applied immediately to 0.5X mutation detection enhancement (MDE) gel (FMC BioProduct Rockland, USA). Electrophoresis was carried out at room temperature in 0.6X TBE at 280 V. Gels were stained with silver staining technique and photographed.

PCR Analysis of TcR β, γ and δ gene rearrangements

T-cell receptor (TcR) gene rearrangements were studied using β, γ and δ chain primers for the V (variable), D (diversity) and J (junctional) regions for T-cell respectively. Purified DNA (250 ng/μl) was added to PCR mix containing 0.2 mM dNTPs, 1.5 mM MgCl2, 68 pmol of primers for β-chain, 50 pmol of primers for γ-chain, 30 pmol of primers for δ-chain and 1.5 U AmpliTaq Gold. Primer sequences, thermal cycling and product analysis were as reported previously. Subsequently heteroduplex analysis was performed exactly as described by Langerak et al.

Results

In a period of 10 years between 1992 and 2001 a total of 1610 cases of NHL (both T and B) were diagnosed in the Department of Pathology, The Aga Khan University Hos-