Abstract

Human Herpesvirus-6 (HHV-6) is ubiquitous in human populations and is the causative agent for exanthem subitum. Primary infection occurs in CD4 T lymphocytes and it is cytopathic in the productive phase of infection. The virus is pleiotropic for several established cell lines including T and B lymphocytes, macrophages and neural cells.

The primary objective of the study was to determine the frequency of HHV-6 DNA sequences in tissue specimens from leukemia and lymphoma, premalignant and malignant oral lesions and cervical carcinoma patients. From this study, DNA was successfully extracted from fresh blood, fresh frozen tissue and archival (formalin fixed) tissue. DNA obtained were from leukemia, oral carcinoma and cervical carcinoma. These DNA were successfully amplified using the Polymerase Chain Reaction (PCR) method for the detection of HHV-6. It has also been observed that a nested PCR protocol was more sensitive in the detection of low copy numbers of HHV-6 DNA. PCR has thus been shown to be useful in HHV-6 viral diagnosis in various types of cancer. Furthermore, a non-radioactive method of detecting amplified products has been optimized and has been found to be advantageous with respect to potential hazards, ease of labelling and long term storage when compared to traditional radioactive methods of probe labelling and hybridization.

Analysis of DNA extracted from leukemia with PCR showed that 3/41 (7.3%) were positive for HHV-6 with outer primers and 16/41 (39.0%) with nested primers. All the 16 nested amplicons hybridized to a digoxigenin labelled HHV-6 specific oligonucleotide. Using another set of primers (A and B) it was noted that 10/41 (24.3%) were positive and all 10 (24.3%) hybridized to another digoxigenin labelled HHV-6 specific

oligonucleotide probe. This suggests that the HHV-6 DNA was present in low copy number and hence the efficiency of detection in nested PCR. Previous data shows high seroprevalence of HHV-6 in leukemia patients, indicative of reactivation of the virus in those patients but the overall low frequency of DNA in the transformed cells suggests that the virus was reactivated independently.

No amplification of HHV-6 DNA sequence was observed with outer primers in 5 lymphoma patients but upon nesting, 1 (20.0%) patient's DNA was positive but with another set of primers(AandB) 2 (40.0%) samples were positive. All 3 samples hybridized with the appropriate probes. Our previous serological data showed that EBV but not HHV-6 was reactivated in lymphoma patients. Thus in these lymphomas the two viruses act independantly and the prevalence possibly suggests that the B-cell pools of cells are involved in EBV related lymphoma.

18 biopsy material of oral carcinoma of the buccal mucosa (fresh frozen tissue and archival tissue) were also obtained and analysed for HHV-6 DNA. Initial amplification with outer primers revealed 6 (33.3%) positive patients but upon nesting 11 (61.1%) patients were positive for HHV-6 DNA. The use of A and B primers in another PCR reaction showed 10 (55.5%) positive patients. All amplified products of both nested PCR and direct PCR hybridized specifically to the P2 and S, Dig labelled probe respectively. In addition, 23 (88.4%) out of 26 paraffin sections from buccal carcinomas stained immunohistochemically to a monoclonal antibody reactive to a late HHV-6 associated protein. The reaction was localized in the cytoplasm and nucleus of the malignant squamous epithelial cells. Control tissues did not react with the monoclonal antibody and antibody specificity was confirmed by virus adsorption studies. Moreover, it was noted that 3 (60.0%) out of 5 premalignant (leukoplakia and lichen planus) were positive for HHV-6 by nested PCR and PCR with

primer A and B. 4 (80.0%) of the samples were also reactive to the monoclonal antibody immunohistochemically.

Since the virus has been shown to have an oncogenic potential in *in vivo* studies, our observation, therefore suggest a possible cofactorial role for HHV-6 in the pathogenesis of oral carcinoma.

From the 19 samples of cervical carcinoma biopsies obtained, only 2 (10.5%) samples were positive by nested PCR to HHV-6 DNA. Both the amplified products hybridized to the P2 probe. No amplification nor hybridization was noted with primer A and B. Furthermore, no protein reactivity to a late HHV-6 associated protein immunohistochemically was noted in the 10 CIN3 and CIS samples obtained. These observations would strengthen the hypothesis that saliva which is present in the oral cavity may be important in the infection of oral epithelial cells.

Thus, like EBV, HHV-6, a ubiquitous virus with an apparent *in vitro* cytopathic activity, show dual tropism for lymphocytes and epithelial cells. Infection of the oral epithelial cells on the other hand may not be cytopathic to the host cell but may have a cofactorial role in the transformation towards the pathogenesis of oral carcinoma.

ABSTRAK

Human Herpesvirus 6 (HHV-6) merupakan virus ubikuitos dalam populasi manusia dan adalah punca utama exanthem subitum. Infeksi primari adalah pada limfosit T CD 4 dan adalah sitopatik dalam fasa produktif infeksi.

Matlamat utama projek ini adalah untuk menentukan frekuensi DNA HHV-6 dalam spesimen tisu dari pesakit leukemia dan limfoma ,pra-kanser dan kanser mulut dan serviks. Dalam projek ini ,DNA dapat diekstrak dari pelbagai tisu termasuk darah, tisu segar, dan tisu terawet. DNA ini juga dapat diamplifikasi dengan teknik rantaian polimeras (PCR).PCR berganda (nested) didapati lebih berkesan untuk diagnosa infeksi HHV-6 dalam pelbagai jenis barah. Tambahan pula kaedah non-radioaktif untuk mengesan DNA yang diamplifikasikan dengan PCR dioptimakan dan juga berkelebihan dari aspek kesan bahaya, kesenangan melabel dan penyimpanan berbanding dengan kaedah tradisi radioaktif.Analisa DNA dari pesakit leukemia dengan PCR mendapat 3/41 (7.3%) positif untuk DNA HHV-6 dengan primer luar dan 16/41 (39.0%) dengan primer dalam. Kesemua 16 amplikon berhibridisasi kepada HHV-6 tertentu (Digoxigenin probe). Pengunaan set primer lain (A dan B) menghasilkan 10/41 (24.3%) positif dan kesemua amplikon juga berhibridisasi kepada HHV-6 tertentu digoxigenin probe lain. Keadaan ini mencadangkan bahawa DNA HHV-6 berada secara berbilang rendah dan menunjukan keberkesanan PCR berbilang . Dari data seroprevalens dalam pesakit leukemia didapati tinggi dan juga menunjukkan reaktivasi virus tetapi frekuensi DNA yang rendah pula mencadangkan bahawa virus ini direaktivasi secara lain.

Tiada amplifikasi didapati untuk DNA HHV-6 dengan primer luar dalam 5 pesakit limfoma teteapi selepas PCR berganda (primer dalam) , 1 (20.0%) pesakit limfoma didapati positif untuk DNA HHV-6 . Dengan set primer lain (A dan B) pula , 2 (40.0%) sampel didapati positif. Ketiga - tiga sampel berhibridisasi kepada probe masing - masing.

Data serologi dulu mendapati bahawa EBV dan bukannya HHV-6 direaktivasikan dalam pesakit limfoma. Dengan ini, dalam pesakit limfoma, kedua-dua virus berinteraksi secara sendirian dan prevalens mencadangkan kumpulan sel B adalah berkaitan dengan limfoma EBV.

18 sampel barah dari rongga mulut khasnya bahagian pipi (termasuk sampel segar dan terawet) didapati dan dianalisa untuk DNA HHV-6. Amplifikasi dengan primer luar memberi 6 (33.3%) positif tetapi setelah PCR berganda 11 (61.1%), pesakit didapati positif untuk DNA HHV-

6. Pengguna set primer lain (A dan B) menunjukkan 10 (55.5%) pesakit yang positif. Kesemua produk amplifikasi hibridisasikan kepada prob masing-masing. Tambahan pula, 23 (88.4%) dari 26 sampel terawet barah mulut didapati positif dengan teknik imunokimia dengan penggunaan antibodi monoklonal yang spesifik untuk protin HHV-6. Reaksi didapati pada sitoplasma dan nukleus sel-sel malignan. Tisu-tisu kawalan tidak bereaksi dengan antibodi monoklonal dan penentuan antibodi monoklonal terbukti dengan penyerapan virus.

Didapati juga 3 (60.0%) dari 5 kes pra-malignan (leukoplakia dan liken planus) positif untuk DNA HHV-6 dengan PCR berganda dan set primer lain (A dan B). Empat (80.0%) daripada sampel ini bereaksi dengan antibodi monoklonal secara imunokimia.

Virus ini didapati mempunyai kesan onkogenik, pemerhatian dari projek ini mencadangkan salah satu punca secara kofaktor untuk HHV-6 dalam perkembangan barah mulut. Dari 19 kes karsinoma serviks, hanya 2 (10.5%) sampel didapati positif selepas PCR berganda untuk DNA HHV-6. Kedua-dua produk amplifikasi berhibridisasi kepada prob P2. Tiada amplifikasi atau hibridisasi didapati dengan set primer lain (A dan B). Tambahan pula tiada reaksi protin HHV-6 dalam sampel terawet pra-malignan atau karsinoma *in situ.* (CIN III/CIS) yang didapati sacara imunokimia.

Pemerhatian di atas, menkukuhkan peranan air liur dalam rongga mulut sebagai faktor penting dalam infeksi sel-sel epithelial rongga mulut. Kini, HHV-6 seumpama EBV adalah virus ubikuitos mempunyai kesan sitopatik yang menunjukkan tropisma dual untuk sel-sel limfosit dan juga sel epitelial. Infeksi sel epitelial mulut mungkin tidak menghasilkan kesan sitopatik kepada sel hos tetapi, mungkin berperanan dalam transformasi sel ke arah perkembangan barah mulut.