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# MOLECULAR DETECTION OF HUMAN HERPESVIRUS-6 INFECTION IN SELECTED CARCINOMAS

by

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# *ABSTRACT*

## ABSTRACT

Human herpesvirus-6 (HHV-6), a CD4<sup>+</sup> lymphoproliferative virus ubiquitous in human populations worldwide, is the causative agent for exanthem subitum, febrile illness and heterophile-negative infectious mononucleosis-like disease. The detection of HHV-6 in several virus-associated cancers suggests a potential copathogenic role in the development of the disease. The aim of the current study was to detect and localize HHV-6 DNA and virus-encoded antigens in various epithelial carcinomas, namely oral, cervical, breast, salivary gland, larynx and nasopharynx. Appropriate controls were incorporated where available. This is the first report of the detection by *in situ* hybridization of the HHV-6 variant A and B types in tissues.

Archival, formalin-fixed and paraffin-embedded carcinoma tissue were examined by polymerase chain reaction (PCR) and nonradioactive *in situ* hybridization (NISH), for HHV-6 A and B DNA. Furthermore, virus-encoded antigens present in the tissues were detected by immunohistochemistry (IHC). Corresponding normal and premalignant tissues were also studied.

Oral carcinoma tissue subjected to PCR, followed by Southern hybridization of amplicons showed that 40.5% cases were positive for HHV-6 DNA. The NISH localized viral DNA in the tumour cells of

78.6% cases and IHC showed the presence of HHV-6 antigens in 88.1%. HHV-6 DNA and antigens were found in 92.3% and 84.6% of premalignant oral lesions by NISH and IHC respectively; 50% of normal oral lesions were HHV-6 DNA and antigen positive by both methods. In salivary gland carcinoma, HHV-6 was found in 33.3% cases by PCR, 85.7% cases by NISH and 100% by IHC. Both PCR and NISH of cervical carcinoma tissue showed the presence of HHV-6 DNA in 33.3% cases while IHC revealed HHV-6 antigens in 46.7% cases. Analysis of normal cervical lesions revealed HHV-6 DNA in 12.5% cases by PCR and NISH while HHV-6 antigen was found in 62.5% cases. HHV-6 was also found in laryngeal and nasopharyngeal carcinoma tissue. However, the virus was not detected in ten breast carcinoma tissue studied.

The high prevalence of HHV-6 detected in oral carcinoma tissue suggests a potential role for the virus in the progression of normal cells to neoplasia. Since HHV-6 DNA and antigens were found in normal and premalignant oral lesions, it suggests a long incubation period with the virus. Thus the virus may act as a cofactor in the multistep carcinogenesis of a subset of epithelial carcinomas.

## ABSTRAK

Human herpesvirus-6 (HHV-6), sejenis virus yang tersebar luas dalam populasi manusia, menyebabkan penyakit kanak-kanak, exanthem subitum dan sering dikaitkan dengan masalah limfoproliferatif. Baru-baru ini, pengesanan HHV-6 dalam kanser oral mengemukakan suatu peranan kopatogenik bagi virus tersebut yang mengandungi turutan gen pengubahsuaian. Matlamat kajian ini adalah untuk mengesan dan memencil jangkitan HHV-6 dalam kanser oral, serviks, payudara, kelenjar liur, larinks dan nasofarinks melalui teknik bukan radioaktif.

Tisu kanser yang ditetap-formalin dan terbenam-paraffin dikaji dengan reaksi rantai polimerase (RRP) dan hibridisasi *in situ* dalam pengesanan DNA HHV-6 dan varian. Kajian imunohistokimia juga dijalankan. Di mana boleh, sampel normal dan pra-kanser telah dikaji bersama.

RRP diikuti oleh hibridisasi Southern, menunjuk 40.5% kes kanser oral dengan DNA HHV-6, hibridisasi *in situ* mengesan DNA virus dalam sel barah 78.6% kes manakala imunohistokimia menunjuk ekspresi antigen HHV-6 dalam 88.1% kes. Kanser kelenjar liur pula menunjuk jangkitan HHV-6 dalam 33.3% kes dengan RRP, 85.7% dengan hibridisasi *in situ* dan 100% dengan imunohistokimia. RRP dan hibridisasi *in situ* tisu kanser serviks mengesan DNA HHV-6 dalam 33.3% kes sementara

imunohistokimia mengesan antigen HHV-6 dalam 46.7% kes. Kehadiran DNA dan protein HHV-6 juga dikesan dalam kanser larinks dan nasofarinks kecuali kanser payudara.

Pengesanan HHV-6 dalam kebanyakan kes kanser oral mencadangkan suatu peranan bagi virus tersebut dalam karsinogenesis. Memandangkan bahawa DNA dan antigen HHV-6 juga ditemui dalam lesi oral normal dan pra-kanser, virus ini mempunyai masa inkubasi yang lama. HHV-6 mungkin berperanan sebagai kofaktor suatu subset kanser, terutamanya oral dan serviks, di mana virus tersebut dapat mengaktifkan onkogen sel dan virus lain.

## LIST OF ABBREVIATIONS

A+T	Adenine and Thymine
ACIF	Anti-complement immunofluorescence assay
ADC	Adenocarcinoma
AEC	3-amino 9-ethylcarbazole
AIDS	Acquired Immunodeficiency Syndrome
$\beta$ -globin	Beta globin
bp	base pair
CFS	Chronic fatigue syndrome
CIS	Carcinoma <i>in situ</i>
cm	centimetre
CoCl <sub>2</sub>	Cobalt chloride
CsCl	Cesium chloride
°C	degree Celsius
dATP	deoxyadenosine triphosphate
ddH <sub>2</sub> O	double distilled water
dig	digoxigenin
dNTP	deoxynucleotide triphosphate
DR	Direct repeat
EBV	Epstein-Barr virus
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ES	Exanthem subitum
fmol	fantomol
G+C	Guanine and Cytosine
gp	glycoprotein
H&E	Hematoxylin and Eosin
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HCl	Hydrogen chloride
HCMV	Human cytomegalovirus
HD	Hodgkin's disease
HEPES	Hydroxyethylpiperazine ethanesulfonic acid
HHV-6	Human herpesvirus-6
HHV-7	Human herpesvirus-7
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV-1	Herpes simplex virus-1
HSV-2	Herpes simplex virus-2
IB	Immunoblot
IE	Immediate early
IFA	Immunofluorescence assay
IHC	Immunohistochemistry



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IM	Infectious mononucleosis
IR	Internal repeat
kb	kilobase pair
kDa	kiloDalton
KU	Kutapressin
LB	Luria Bertani
LiCl	Lithium chloride
LTP	Large tegument protein
LTR	Long terminal repeat
M	Molar
MAB	Monoclonal antibody
MCP	Major capsid protein
MDBP	major DNA binding protein
Mg	Magnesium
$\mu$	micron
$\mu$ g	microgram
$\mu$ l	microlitre
$\mu$ M	micromolar
mg	milligram
MgCl <sub>2</sub>	Magnesium chloride
ML	malignant lymphoma
ml	millilitre
mM	millimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	nanogram
NHD	Non-Hodgkin's disease
NISH	Nonradioactive <i>in situ</i> hybridization
nm	nanometer
NPC	Nasopharyngeal carcinoma
OBP	Origin binding protein
ORF	Open reading frame
%	percentage
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
pmole	picomole
RNase	Ribonuclease
rpm	revolutions per minute
SCC	Squamous cell carcinoma
SDS	Sodium dodecyl sulphate
TBE	Tris-borate-EDTA
U	Unit
UL	Unique long

UV	Ultraviolet
V	Voltage
v/v	volume per volume
VZV	Varicella-Zoster virus
w/v	weight per volume
WHO	World Health Organization

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