INTRODUCTION

CHAPTER ONE: INTRODUCTION

Human herpesvirus-6 (HHV-6) was initially isolated from the peripheral blood mononuclear cells (PBMC) of patients with AIDS and lymphoproliferative disorders (Salahuddin *et al.*, 1986). The virus has since been reported ubiquitous in populations worldwide (Pellett *et al.*, 1992) (Table 1.1). Based on close similarities to HCMV, the virus is recognized as a betaherpesvirus (Lawrence *et al.*, 1990; Neipel *et al.*, 1991; Nicholas and Martin, 1994).

HHV-6 appears to be transmitted via saliva (Gopal *et al.*, 1990; Harnett *et al.*, 1990; Levy *et al.*, 1990) but other sources have been suggested, including urine, blood and cervical fluids (Luka *et al.*, 1990; Asano *et al.*, 1991b, Leach *et al.*, 1994). The virus has a primary tropism for $CD4^+$ T lymphocytes (Lusso, 1987), but it has been observed to infect a variety of cells, including B lymphocytes (Ablashi *et al.*, 1988a), epithelial cells (Fox *et al.*, 1990, Krueger *et al.*, 1990; Chen *et al.*, 1994a) and other cells under certain circumstances (Ablashi *et al.*, 1988b, Lusso *et al.*, 1987).

HHV-6 can be classified into 2 variant types, designated A and B based on biological, immunological and molecular studies (Ablashi *et al.*, 1991; Aubin *et al.*, 1991; 1993; Dewhurst *et al.*, 1992). HHV-6B

Table 1.1: Classification of Human Herpesviridae and their seroprevalence in human populations [(a)]

Human Herpesviridae	**Classification**	**Seroprevalence in healthy populations**
Herpes Simplex Virus-1 (HSV-1)	α	90%
Herpes Simplex Virus-2 (HSV-2)	α	70%
Varicella-Zoster Virus (VZV)	α	80%
Human Cytomegalovirus (HCMV)	β	40-60%
Epstein-Barr Virus (EBV)	γ	95%
Human Herpesvirus-6 (HHV-6)	β	35-80%
Human Herpesvirus-7 (HHV-7)	?	95% [(b)]
Kaposi's Sarcoma-associated Herpesvirus (KSHV) / HHV-8	γ	20%

([(a)]Yadav, 1991; personal communication; [(b)]Ablashi *et al.*, 1995c)

has been further clustered into group 1 and group 2 (DiLuca *et al.*, 1992; Chou and Marousek, 1994).

HHV-6 is the aetiological agent of exanthem subitum (ES) (Yamanishi *et al.*, 1988), some cases of febrile illnesses in children (Pruksananonda *et al.*, 1992) and heterophile-negative infectious mononucleosis-like illness (Krueger *et al.*, 1988; Krueger and Sander, 1989). Primary virus infection occurs in the first year of life and then the agent persists, like other herpesviruses, in a latent state throughout the life of the host. HHV-6 has been reported to be reactivated in various diseases including lymphomas (Jarrett *et al.*, 1988; Torelli *et al.*, 1991), chronic fatigue syndrome (Ablashi *et al.*, 1988a), oral carcinoma (Yadav *et al.*, 1994), cervical carcinoma (Chen *et al.*, 1994b; Wang *et al.*, 1994a; Yadav *et al.*, in press) and others (Krueger and Sander, 1989; Pellett *et al.*, 1992; Lusso and Gallo, 1995). The relevance of HHV-6 with these associations remains to be determined although it has been suggested that HHV-6 may be a cofactor in the progression of these diseases.

The observation of the high prevalence of HHV-6 in oral carcinoma prompted the development of the present study. The presence of HHV-6 in mucosal areas in general has been poorly investigated. Certainly little is known of the distribution of HHV-6 in the oral, larynx, nasopharynx and cervical tissues and the present study attempts to address

these issues. Various molecular detection techniques have been employed in the study of HHV-6 infection. This study entails the use of nested PCR to detect and amplify viral DNA sequences and to type the HHV-6 infection by restriction analysis. Nonradioactive *in situ* hybridization and immunohistochemistry were carried out on carcinoma tissue to localize the source of viral infection by detection of HHV-6 DNA sequences and proteins. This is the first report to describe an *in situ* hybridization technique for the detection of HHV-6A and HHV-6B using variant-specific oligonucleotide probes.

The main objectives of this study were :

- to optimize a nonradioactive technique for the molecular detection of HHV-6 DNA in archival, formalin-fixed and paraffin-embedded tissue.

- to detect, using PCR and *in situ* hybridization techniques, the prevalence of HHV-6 A and B genotypes in carcinoma tissues, from the oral and cervical areas.

- to determine the expression, with monoclonal antibodies, of HHV-6 proteins in archival oral and cervical carcinomas by immunohistochemical technique.

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