

# Quantification of Human Papilloma Virus 16 and 18 in Saliva of HIV-infected Patients by real time PCR.

V.Thonier<sup>1</sup>, E.Opoix<sup>2</sup>, M.Blanc<sup>2</sup>, A.Bosseray<sup>3</sup>, G.Bargues<sup>1</sup>, D.Seigneurin<sup>4</sup>, P.Leclercq<sup>2</sup>, J.M.Seigneurin<sup>1</sup>, P.Morand<sup>1</sup>.

Laboratory of Virology<sup>1</sup>, AIDS clinic<sup>2</sup>, Internal medicine departement<sup>3</sup>, Laboratory of cytology<sup>4</sup>, Grenoble University Hospital, Grenoble, France.

**Abstract**

**Background:** Ano-genital infection with high risk 16 and 18 papilloma viruses is frequent in HIV-infected patients, but little is known about the prevalence of oral human papilloma virus (HPV) infection during HIV infection. Our study assessed the presence of HPV16 and HPV18 in saliva of HIV-infected patients by real-time quantitative polymerase chain reaction (PCR).  
**Method:** In an observational study, 130 HIV-positive individuals (63 men, 67 women) were prospectively enrolled. All patients gave blood and oral samples (saliva and cytobrush). Among them, 38 women underwent an additional cervical smear for cytological and virological tests. In-house real-time PCR (Light Cycler®) was performed by amplification of the region E6 (HPV16) and E7 (HPV18). Quantification was carried out by an external standard curve obtained by serial dilutions of recombinant plasmids. β globin gene co-amplification was used as an internal control. The threshold of detection was 10 copies of HPV/500 nanogrammes (ng) of DNA.  
**Results:** Of the 130 patients, 17 (13%) had HPV16 or HPV18 in oral samples (14 HPV 16 and 3 HPV18). Oral HPV loads ranged from 10 copies to 10<sup>6</sup> copies/500 ng of DNA. All blood samples were negative for HPV DNA. Among the 38 women with oral and cervical samples, 17 (45 %) were positive for HPV16 or HPV18 in the genital area (11 HPV16, 1 HPV18, 5 HPV16/18 co-infections). Genital HPV loads ranged from 10 copies to 2.10<sup>7</sup> copies/500 ng of DNA. Four out of 38 (11 %) excreted HPV16 (3) or HPV18 (1) in saliva with low viral loads (< 400 copies/500 ng of DNA) and without visible oral lesion. The 4 corresponding cervical samples showed an abnormal cytology (low-grade squamous intraepithelial [LSIL] or koilocytes) with higher genital HPV viral loads than women without HPV detection in saliva. The presence of oral HPV was not correlated with HIV status (Centers for Disease Control classification and plasmatic viral load) but patients positive in saliva had lower CD4 cell counts than patients with no oral HPV detection.  
**Conclusion:** We found a higher prevalence (13%) of oral HPV 16-18 in HIV-infected patients than the 6% previously reported, probably due to epidemiological or technical differences. Women with HPV in saliva showed a higher HPV load in genital area than women without HPV in saliva. The natural history of oral and genital HPV infections in HIV-infected patients requires further study to understand the relationships and the consequences of this bipolar infection.

**1 Background and Aims of the study:**

- > HIV positive individuals are known to be more frequently infected by High Risk Oncogenic Papillomavirus (HR-HPV) in the anogenital area than HIV free individuals. HIV positive patients present a higher level of anogenital dysplasia and a higher anogenital cancer risk. (1) HR-HPV have also been implicated in head and neck tumors in immunocompetent individuals (2) but little is known about the natural history of oral HPV infection during HIV infection.
- > Our aims were :
  - To assess the prevalence of HPV16 and 18 in saliva of HIV-infected patients by real time quantitative PCR.
  - To evaluate a relationship between oral and genital presence of HPV 16 and HPV 18.

**2 Patients and Method:**

**Observational study:**  
 130 HIV+ individuals (63 men, 67 women) prospectively enrolled (Jan-Oct 2004).  
 All patients gave blood and oral samples (saliva and cytobrush). Among them, 38 women underwent additional cervical smear for cytological and virological tests.  
 Extraction carried out by QIAamp®DNA MiniKit QIAGEN ®.  
 In house real time PCR (Light Cycler®) performed by amplification of the region E6 (HPV16) and E7(HPV18).(Fig 1)

**Primers and Probes :**

- HPV 16
  - 16 as 5'-AGC TCT GTG CAT AAC TGT GGT AAC TT-3'
  - 16 s 5'-TTT TAT GCA CCA AAA GAG AAC TGC-3'
  - 16Taq 5'-ATG TTT CAG GAC CCA CAG GAG CGA CC-3'
- HPV 18
  - 18 s 5'-GCA TTT AGA GCC CCA AAA TGA A-3'
  - 18 as 5'-CGT TTT CTT CCT CTG AGT CGC T-3'
  - 18 Taq 5'-TCC GGT TGA CCT TCT ATG TCA CGA GCA-3'

Quantification carried out by an external standard curve obtained by serial dilutions of recombinant plasmids. βglobin gene co-amplification used as an internal control. The threshold of detection was 10 copies of HPV/ 500 nanogrammes (ng) of DNA.

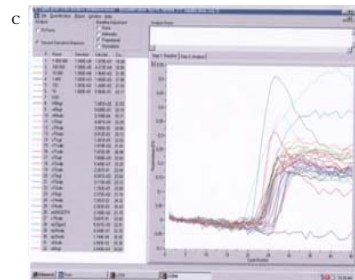
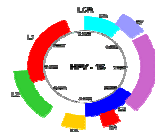


Fig 1: HPV DNA detection by real time PCR (Light Cycler® technology)  
 A/ Patients .B/ Standard curve and positive /negative controls  
 C/ detection of β-globin as an internal control



Tab 1: HPV's prevalence and viral loads in saliva, blood and genital cavity.

Virus	Saliva n = 130	Cervical n = 38	Blood n = 130
HPV 16	14 (10.7%)	16 (42%)*	0 (0%)
Viral loads Range Copies of HPV/500 ng of DNA	10 to 100,000	10 to 20,000,000	
HPV 18	3 (2.3%)	6 (16%)*	0 (0%)
Viral loads Range Copies of HPV/500 ng of DNA	10 to 8,000	10 to 90,000	
HPV 16 or 18	17 (13%)	17 (45%)*	0 (0%)
Viral loads Range Copies of HPV/500 ng of DNA	10 to 100,000	10 to 20,000,000	

\* 5 women were co-infected by HPV 16 and HPV 18

**Result 2**

In the sub-group of the 38 women with genital and saliva samples, the detection of HPV 16 or 18 in saliva was more frequent among women with positive HPV cervical samples. (Table 2).

**3 Results**

**Result 1**

- The prevalences of HPV 16 or 18 in oral and genital areas were 13 % and 45%, respectively (Table 1).
- HPV 16 infections were more frequent than HPV 18 infections in both cavities . HPV 16 viral loads were higher than HPV 18 viral loads.
- Cervical viral loads were higher than oral viral loads.
- All blood samples were negative.

Tab 2 Prevalence of oral HPV status depending on cervical HPV status

saliva	cervix	HPV 16 or 18 positive	HPV 16 or 18 negative
HPV 16 or 18 positive	3	18%	5%
HPV 16 or 18 negative	14	82%	95%
total	17	100%	21
			100%

Tab 3 Genital HPV mean viral load among women with positive or negative HPV detection in saliva

Oral HPV status	Genital HPV 18 mean viral load Copies/500 ng of DNA	Genital HPV 16 mean viral load Copies/500 ng of DNA
Patients positive for oral HPV	700,000	5,000,000
Patients negative for oral HPV	3,500	765,000

**Result 3**

All the four women HPV positive in saliva had a Low grade Squamous Intraepithelial Lesion (LISL) cervical cytology. They presented a higher HPV mean viral load in the genital area than women with no oral HPV detection (Table 3).

**Result 4**

The presence of oral HPV was not correlated with HIV status(CDC classification and HIV viral load) but patients positive in saliva had lower CD4 cell counts than patients with no oral HPV detection. (384 vs 531/mm<sup>3</sup>; data not shown).

**4 Conclusion**

We found a higher prevalence (13%) of oral HPV 16-18 in HIV infected individuals than the nearly 6% previously reported by Kreimer (3), probably due to technical or epidemiological differences. Women with HPV in saliva were also often positive in the genital cavity and showed a higher genital HPV mean viral load than women without HPV in saliva. The detection of oral HPV seems to be correlated with lower CD4 cell counts (3).

The natural history of oral and genital HPV infections in HIV infected individuals requires further studies to understand the relationship and the consequences of this bi-polar infection.

5 References  
 1- Palefsky J, Holly E. *Chapter 6: Immunosuppression and Co-infection*. JNCI Monographs No 31, 2003  
 2- Smith E, Ritchie J. *Age, Sexual Behaviour and Human Papillomavirus Infection In Oral Cavity and Oropharyngeal Cancers*. Int J cancer:108, 765-772 (2004)  
 3- Kreimer A et al. *Oral Human Papillomavirus Infection In Adults Is Associated with Sexual Behavior and HIV Serostatus*. JID 2004; 189: 686-698