P0005
Paper Poster Session
Viral molecular epidemiology (other than Hepatitis/HIV)

Viral load and genomic integration of hman papillomavirus type 16 in oral pre-malignant and malignant disorders

Alessandra Pierangeli*[1], Fabiana Cannella[1], Isabella Calicchia[1], Ilaria Sciandra[1], Massimo Gentile[1], Gaspare Palaia[2], Umberto Romeo[2], Chiara Ciolfi[2], Guido Antonelli[3]

[1]*Sapienza University, Molecular Medicine, Rome, Italy*

[2]*Oral and Maxillofacial Sciences, Rome, Italy*

[3]*Sapienza University of Rome, Department of Molecular Medicine, Rome, Italy*

Background: The prevalence of oral Human Papillomavirus (HPV) infection has significantly increased in the last decade, raising concerns about the HPV role in progression of oral potentially malignant disorders (OPMD) toward squamous cell carcinomas. Our recent study (Pierangeli et al, Clinical Microbiology and Infection, 2015) on oral HPV infection detected unexpectedly high rates of HPV infection in cells of the oral mucosa, with elevated high-risk (HR) HPV loads. To gain insight into this issue, we sought to determine DNA loads and viral integration status in HPV 16 positive OPMD and oral carcinoma.

Material/methods: A total of 80 patients attending Odontostomatologic Clinics, affected by suspected HPV lesions, OPMD or oral carcinomas, were consecutively enrolled in the period January 2013 to October 2015. Oral cells were collected from patient's lesions using a Cytobrush to obtain a large number of transepithelial cells. Total DNA was extracted and tested amplifying the housekeeping gene GAPDH. HPV was detected with quantitative real-time PCR (qPCR) targeting E6 region for the low-risk HPV6,11 and the HR 16,18,31,33,53,58 genotypes. HPV16 positive DNA were subjected also to a qPCR targeting E2 region. The integration status was determined by measuring the E2 to E6 ratio.

Results: From enrolled patients, 40/80 samples were HPV positive; among these, HPV16 resulted to be the most frequent genotype (20 cases, one in coinfections with HPV 31). Median values of HPV16 DNA loads in oral lesions are reported in the table.

LESION	Median values (range) of HPV16 load (copies/ng total DNA
Non-OPMD lesions	$1.2x10^5$ ($1.2x10^3$-$1.3x10^6$)
Leukoplakia	$6.5x10^4$ ($2.7x10^3$-$1.9x10^5$)
Lichen planus	$4.5x10^4$ ($4.3x10^3$-$1.5x10^5$)
Oral carcinoma	$3x10^3$ ($7.3x10^1$-$1.1x10^4$)

Interestingly, HPV 16 DNA resulted integrated in the cell genome at least partially (E2/E6 ratio < 0.5) in 8/21 cases. Among those, all three HPV16-positive carcinoma cases had an integrated viral genome whereas HPV16 genome was integrated in 1/6 HPV16-positive lichen planus. Among other oral lesions, in 4/10 HPV16-positive cases, E2/E6 ratio was <0.5. Integration status correlated well with results of histopathologic diagnosis of the oral lesions.

Conclusions: Our previous study suggested that HPV at elevated viral loads, such those we found particularly in HPV16 and 18 oral infections, could be associated with an increased risk of viral persistence, a key factor in cangerogenesis. In this report, we add the finding that, in biopsy confirmed carcinomas of the oral cavity, HPV 16 DNA was integrated in the cell genome, thus suggesting a role in oral cancer development. Differently, in lichen planus considered an OPMD, HPV 16 DNA integration was far less frequent. Of note is the rate of HPV16 integration in non-OPMD lesions, not referred for biopsy. Prospective studies are needed to establish whether HPV integration may represent a useful marker to identify lesions to be taken for histopathological diagnosis.