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LETTER TO THE EDITOR

Human papillomavirus frequency in oral epithelial lesions

Lazzari et al. (1) investigated the human papillomavirus (HPV) prevalence in different types of oral mucosal lesions, by means of the polymerase chain reaction (PCR) method with MY09/MY11 primers. They reported a general HPV prevalence in their samples of 11.3% (nine of 80): genotypes detected were HPV-6b (two lesions), MM4 (one lesion) and MM9 (one lesion); in the remaining five lesions the HPV type could not be identified, because of DNA degradation. The authors concluded that their findings are quite relevant.

Taking into account the current knowledge on oral HPV infection, we think opportune to make some considerations. Nowadays, in fact, there are increasing evidences supporting the role of HPV in the development of squamous cell carcinoma of the head and neck (SCCHN): recently, some large sample size studies (2–7) showed that SCCHN and potentially malignant oral lesions contain DNA of high-risk HPV genotypes (e.g. HPV-18 and -16).

A first uncertainty in this study is that all potentially malignant lesions investigated (five cases of oral lichen planus and seven of oral leukoplakia) were not biopsied and, hence, not histologically confirmed in their clinical diagnosis.

Moreover, the HPV DNA detection method used in this analysis was MY-PCR, which is not a very sensitive assay, as its limit is 100–200 HPV DNA copy. However, because of the fact that oral HPV infections are weakly productive (7, 8), a more sensitive PCR system is required to obtain reliable information on HPV presence in oral samples with low copy number of viral DNA. To this aim, a highly sensitive nested-PCR assay with MY09/MY11 and inner GP5+/GP6+ primers (7, 8) could be the technique of choice. In addition, the authors provided scant information on the MY-PCR protocol used; in particular, they did not indicate whether they used TaqGold polymerase, which identifies about 50% more infection by high-risk HPV types compared with *Taq* polymerase; also, the cycling parameters of amplification were not described. The method of diagnosis is a critical issue, since methods with limited sensitivity of HPV detection may misclassify important viral infection patterns and results in artefacts. In this context, it should be noted that in the paper Lazzari et al. the HPV detection rate in each group of lesions is very low. In a recent meta-analysis on HPV oral infection (9), HPV DNA was evident in 22.2% benign leukoplakias, 26.2% intraepithelial neoplasias and 46.5% oral squamous cell carcinoma; differently, Lazzari et al. did not find any HPV-positive oral carcinomas and detected only one case of HPV-positive oral lichen planus. Additionally, neither HPV-16 nor HPV-18 or the other high-risk HPV types usually detected in oral mucosal lesions have been detected in samples from this assay, and this finding is also surprising. Unfortunately, they did not either reported frequencies of HPV infection in any control group or comparisons with literature data.

Finally, the datum reported and commented by the authors, i.e. HPV DNA detection frequency in all lesions, analysed as a whole, is not very informative. Indeed, it refers to a group of very different clinical lesions: of these, some are benign lesions (namely papillomas), supposed to be HPV-related, while others are potentially malignant (oral lichen and leukoplakia) or frankly malignant (oral squamous cell carcinoma) lesions, still under study for their possible association with HPV infection. Reporting generic HPV prevalence in different types of oral lesions is not very helpful to evaluate the role of HPV as a possible risk factor for pre-cancerous or cancerous oral lesions. It would have been more interesting to focus on HPV detection rate in each different group of lesions, also to compare author findings with literature data.

Therefore, in our opinion, although the report of two uncommon HPV genotypes in oral lesions, it is very difficult from this findings to reach to informative or relevant conclusions.

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Reply to 'Letter to the Editor'

Thank you for giving us more time to answer the comments made to our paper: Human papillomavirus frequency in oral epithelial lesions [Lazzari et al. (1)]. First, we would like to clarify that we have planned a study using material from smears collected with a cytobrush and not from biopsied lesions. Our main objective was to determine the frequency of human papillomavirus (HPV) in these lesions (only in oral epithelial lesions). It should be pointed out that the use of this technique of collecting material maintains a percentage viability similar to what is found when using DNA extraction of biopsied material (2). In addition, not all lesions were biopsied because this is not a routine in the Stomatology Ambulatory where the samples were collected. In fact, the routine involves taking biopsies only when it is necessary to remove the total lesion or when the clinical characteristics do not allow a clear diagnostic definition. The biopsed samples that were no epithelial lesion were excluded from the analysis.

The use of specific MY primers of the L1 region of the HPV genome was already established in our laboratory, and currently are indeed too many scientific manuscripts supporting their utilization with confident results. Beside that, in our laboratory the use of the GP5+/GP6+ system is not yet standardized, by the contrary, in some doubtful cases of HPV infection, the utilization of these primers showed less sensitivity and as above-mentioned the MY primers were the available tools at the moment of our study. Sure, more sensitive primers are currently being assayed. Indeed, primer sets such as SPF1/2 were tested even with better results than the GP5+/6+. On the contrary, it is important to point out that the standardization of the GP5+/6+ has several difficulties in standardization procedures, because the internal adapter in their long sequence.

Regarding the utilization of a more purified *Taq* DNA polymerase as suggested, we can mention that as the purification procedures were very carefully conducted, the amplification was performed as in our laboratory routine assays, we do not consider as a crucial point the utilization of enzymes of better quality, because as demonstrated in another manuscripts, the utilization of any other one is enough to get excellent results.

As it was mentioned in the article, the literature describes a large range of HPV-positive frequencies in oral lesions (from 5 to 80%) (3–5). A low positive frequency observed in our sample was also surprising to our team, specially because we were expecting a low income population with less access to health services for regular visits. However, these characteristics in our studied population were evenly distributed. Therefore, we believe that this fact contributed for the lower HPV frequency reported in our article. Also, the observed HPV frequency is within the large range described in the literature.

It should also be mentioned that one of the limitations of our work was the small size of the studied sample. This fact made difficult to analyse the HPV positivity stratified into different lesion types, i.e. there was no sufficient individuals in each cell to perform the analysis. In fact, we observed: five lesions of lichen planus (one HPV+, corresponding to 20% within these lesions); seven lesions of leukoplakia (one HPV +, corresponding to 14.3% within these lesions). These frequencies are similar from the results reported by Giovannelli et al. (6). We have considered that the number of cases in each strata observed in our study was small to make further statistical analysis.

Another limitation of our work was the fact that we did not carry out the sample collections in duplicate which would have been very helpful to prevent the loss of material because of degradation.

Considering all comments and despite the limitations of this work, we believe that the publication of our results are relevant to the scientific community even with the low HPV frequency observed, specially because of the atypical HPV types found in the oral lesions.

We thank you again for giving us more time to answering the letter, and we hope that our comments are within the expectations of Dr Giovannelli's team.

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