Reviewer’s report

Title: High-risk human papillomavirus (HPV) screening and detection in normal, healthy patient saliva samples: a pilot cluster randomized study.

Version: 1 Date: 2 February 2011

Reviewer: Ruth Tachezy

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The manuscript by Turner et al. entitled „High-risk human papillomavirus (HPV) screening and detection in normal, healthy patient saliva samples: a pilot cluster randomized study“ deals with the prevalence of HPV-16 in the saliva specimens of healthy adults.

Altogether 102 specimens of 151 enrolled subjects were evaluated by means of type-specific PCR for the presence of HPV-16. The specificity of the reaction was confirmed by quantitative real-time PCR with type-specific primers targeted to E6 region of HPV-16.

Major comments:

The major aim was to assess HPV prevalence in healthy adults in Las Vegas, Nevada, USA. None-invasive method of saliva sampling has been used. In this state recently increasing rate of oral cancer has been documented despite the decline in the rates of well known risk factors – smoking and alcohol consumption. The authors claim this as a pilot study.

Background

The introduction is unnecessary long. It needs to be more focused. I would also suggest including studies done by using oral lavage as none-invasive method of specimen collection on healthy controls in the introduction (see Herrero et al., 2003; Smith et al., 2004; Koppikar et al., 2005; Zhao et al., 2005; D'Souza et al., 2007; Tachezy et al., 2009).

Some parts of the introduction are quite confusing (e.g. The role of HPV in the oral cavity, however, may differ by anatomic site and also by the particular strain of HPV infection [35]. For example, low-risk HPV strains 6 and 11 have been identified in benign laryngeal papillomas, common warts (verruca vulgaris), and condyloma acuminatum [36-38]). It is important to distinguish studies by anatomical site. Laryngeal papilloma is not disorder of the oral cavity.

In the sentence below the localization of tumors has to be specified (underlined). Recent epidemiologic and case-control studies have demonstrated that patients with HPV-positive tumors had significantly better response rates to chemotherapy and chemoradiation treatments when compared with HPV-negative tumors [28,46-48].

Methods
No information of controls of eventual carry over contamination during the extraction of DNA as well as incorporation of positive and negative controls for PCR reactions are provided. The region which targets primers used for qualitative PCR is not specified. For quantitative PCR information about the primers and method in general is missing. Also, no information of construction of a calibration curve for HPV-16 quantification is provided.

Results

Expression of statistical significance is rather unusual (p < 0.1, p > 0.05), either exact value or information - significant or not significant, should be used.

The numbers if in the middle of sentence, can be expressed as numbers not by words, e.g. 102, or even better 102/151 specimens.

The use of quantitative PCR for this study gives no additional value. Since primers for both types of PCR were targeted to E6 region, the qualitative PCR should detect also the integrated form of HPV-16 and therefore not increase the number of false negative samples. Furthermore, only the positive samples and 10 negative samples on the qualitative PCR were rescreened by quantitative real time PCR. The later method is more sensitive and therefore in theory it can detect higher prevalence of HPV. The use of this method would make sense only if all samples are tested. However, as it is presented in this study it has no value. Also the information about the number of copies below the cutoff limit is redundant.

Since this study is aimed on assessment of HPV prevalence in the healthy population the method of detection of multiple HPV types or at least multiple HR HPV types would be more appropriate.

In conclusion the presented data are truly pilot data. The HPV prevalence should be tested by a method which allows detection of multiple HPV types and is also used in some other studies to allow for comparison. The study would greatly benefit from enlarging of the number of subjects to be able to perform detailed statistical analyses and questionnaire for assessing possible risk factors which can eventually modify the presence of HPV in the oral cavity of healthy adults.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.