Reviewer's report

Title: Screening and detection of human papillomavirus (HPV) high-risk strains HPV16 and HPV18 in healthy pediatric patient saliva samples from Nevada: a pilot study.

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Reviewer: Ruth Tachezy

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The manuscript by Flake et al. entitled „Screening and detection of human papillomavirus 1 (HPV) high-risk strains HPV16 and HPV18 in healthy pediatric patient saliva samples from Nevada: a pilot study“ deals with the prevalence of HPV-16 and -18 in the saliva specimens of healthy children and teenagers. Altogether 118 specimens were evaluated by means of type-specific PCR for the presence of HPV-16 and -18. The specificity and sensitivity of the reaction was confirmed by quantitative real-time PCR with type-specific primers targeted to E6/E7 region of HPV-16.

Major comments:

The major aim was to assess HPV prevalence in healthy children and teenager in Las Vegas, Nevada, USA. None-invasive method of saliva sapling 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.

Major Compulsory Revisions

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.

Background

The background is focused on information about HPV and cervical and head and neck cancer. Since the prevalence of HPV in healthy children was studied, entirely different information should be included in the introduction, etc. prevalence of HPV in young people and adults, comparison of the age specific prevalence, differences caused by different methods used…..

Page 3: Although high risk HPV drives the transformation and malignancy process of nearly all cervical adenocarcinomas… and how about squamous cell carcinoma of cervix?

Page 3: The higher prevalence of high-risk HPV strains in pre-cancerous and cancerous oropharyngeal tumors suggests that HPV may preferentially infect developing or established cancers, thereby modulating carcinogenic progression
and ultimately influencing patient outcomes. Do the authors mean that in those patients with head and neck cancers who get infected by HPV, the prognosis improves? So HPV is not etiological factor of a subset of head and neck cancers? Isn’t HPV the starting point of immortalization and transformation of epithelial cells?

In addition, new evidence now suggests that some high-risk HPV strains, such as HPV16 and HPV18, may initiate oral carcinogenesis among the smaller fraction of oral cancer patients who do not consume alcohol or use tobacco. The etiological relationship is accepted for HPV 16 and oropharyngeal cancer.

Page 4: To this end, less invasive methods, such as oral lavage- and saliva-based screening have yielded significant results. What the authors mean by significant results?

Results

The authors specify in details the amount of DNA gained from saliva’s, but since internal control for PCR and housekeeping gene for qPCR was used it is irrelevant information.

Why didn’t the authors used also primers for HPV 18? How the authors confirm the sensitivity of PCR when qPCR was type specific for HPV 16 only and one sample contained HPV 18?

Why didn’t the authors used system which allows for the detection of a wide number of HPV types when prevalence in healthy individuals was studied?

Materials and Methods

Please specify in more details the process of sampling.

Why did the authors count the cells when they didn’t use a fixed input number for PCR?

Figure 1 is redundant.

**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.