Dear Editor,

During a literature search about the role of molecular markers in accurate diagnosis and prognosis of oral malignant lesions, we have encountered a valuable paper entitled “Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population”, authored by Brinkmann O, Kastratovic DA, Dimitrijevic MV, Konstantinovic VS, Jelovac DB, Antic J, Nesic VS, Markovic SZ, Martinovic ZR, Akin D, Spielmann N, Zhou H, Wong DT, published in Oral Oncology 2011 January; 47(1): 51–55. In that study, the authors investigated six transcriptome (DUSP1, IL8, IL1β, OA21, SAT1, S100P) and three proteome (IL1β, IL8, M2BP) biomarkers on 18 early and 17 late stage OSCC patients and 51 healthy controls with quantitative PCR and ELISA. Their results showed that four transcriptome (IL8, IL1β, SAT1, S100P) and all proteome biomarkers were significantly elevated (p < 0.05) in OSCC patients and that a combination of markers from the proteome and transcriptome yielded the highest predictive power for OSCC (IL1β protein + SAT1 mRNA + DUSP1 mRNA) with an AUC of 0.86, 0.89 sensitivity, and 0.78 specificity. Those OSCC salivary biomarkers were not dependent on the ethnicity of the patients and were presented as a highly promising approach for OSCC detection.

Although oral squamous cell carcinoma (OSCC) is the most frequent malignancy in oral cavity and many methods have been developed for early recognition, most lesions are still detected in advanced stages [1–6]. Late stage detection is thought to be one of the reasons for lower 5-year survival rates compared to early stage disease [7].

OSCC may be preceded by noticeable precursor lesions which are defined as oral potentially malignant disorders (OPMDs) [5,8–12]. Identification of OPMDs with higher risk of malignant transformation is one of the major challenges in prevention trials and patient care. Most of the studies on OSCC tumor markers performed in serum have been replicated in oral fluids, including whole unstimulated saliva or oral rinses. Among the methods to detect malignant transformation risk, molecular evaluation of saliva has been suggested as an easy, non-invasive potential means [6,7,13–20].

Saliva contains constituents of exocrine glands in the oral cavity and gingival crevicular fluid, that incorporates constituents that reveal the diseased or physiological state of the host, and hence present the potential to be utilized for diagnostic purposes [18,19,21–24]. As saliva is readily available, does not require specialized equipment for collection and is in direct contact with oral mucosa/oral cancer, the search for reliable salivary biomarkers for early detection and prediction of behavior of OPMDs and of OSCC for planning cancer therapies and predicting prognosis of cancer has developed rapidly [6,7,14,18–20,25]. However, the carcinogenic process, the fluid manipulation, standard saliva collection, processing and storage procedures, and the highly variable composition of saliva between patients must be known in order to fully understand the value in detection and in diagnosis of oral malignancies. One of the biggest challenges is to distinguish benign lesions from potentially malignant lesions, and dysplastic/malignant ones from inflammatory conditions. For this purpose, the patients with chronic oral inflammatory diseases must be differentiated from those with oral premalignant disorders or oral cancers [7,25]. As observed in Table 1, out of thirteen studies which evaluated the role of salivary protein biomarkers in OSCC detection, only four papers have taken the presence of chronic periodontitis into consideration [6,14,15,25–35].

Chronic periodontitis is the most common disorder of the oral cavity in adults, which is caused by predominantly pathogenic microorganisms that colonize the subgingival area and trigger local and systemic elevations of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8 resulting in tissue destruction [25,36,37].

To date, several mediators of chronic inflammation and tissue destruction have been detected in whole saliva of both OSCC and periodontitis patients [13,15,21,38]. Salivary total protein levels, matrix metalloproteinases (MMPs), IL-6 and IL-8 are among the most studied biomarkers, both for OSCC, OPMDs and chronic periodontitis [14,15,21,27–30,33–35,39–41]. Metalloproteases, mostly MMP-2 and MMP-9, participate in cancer pathogenesis and periodontal destruction by degrading type-IV, V, VII and X collagen and elastin and fibronectin [30,32].

These inflammatory cytokines, IL-6 and IL-8, are intercellular signaling proteins which regulate the growth, cellular proliferation, angiogenesis, tissue repair and also function in immune responses to infection, injury and inflammation [42]. Thus, it is challenging to evaluate the impact of one cytokine or cytokines in an event at the cellular level because of the complexity of its interactions with each other and other tissues [42]. Also, several conditions such as rheumatoid arthritis, periodontal disease, osteoporosis, Sjögren syndrome, chronic parotitis, severe exercise and diabetes can give rise to increased levels of inflammatory proteins [14]. As a result, combination of molecular markers has been discussed for use in detection and diagnosis of OPMDs and OSCC. Significant alteration of salivary components was observed in OSCC, indicating a compromised oral environment in oral cancer patients [30]. Similarly, high levels of both sCD44 (>14.56 ng/ml) and protein (>0.4325 mg/ml) were associated with HNSCC [6]; subjects with high soluble CD44 and total salivary protein levels were estimated to have cancer almost 25 times more than those without these elevated levels (odds ratio [OR] = 24.90; 95% confidence interval [CI] = 9.04–68.57) [6].

In early OSCC diagnosis studies, wide variations in the salivary IL-6 and IL-8 levels among different studies were noted even in the healthy controls, with the average IL-6 levels ranging from

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1.4 ± 0.9 pg/ml [25] to 47.46 ± 18.74 pg/ml [28], and IL-8 levels ranging from 250 pg/ml [27,29,31] to 1945 ± 181 pg/ml [38]. It is obvious that such a wide spectrum of reference levels impairs the determination of salivary IL-6 or IL-8 levels to indicate OSCC development. Additionally, the average salivary IL-8 levels in chronic periodontitis (2268 ± 111 pg/ml) and healthy controls (1945 ± 181 pg/ml) were within the range reported for OSCC [27,31,38].

Despite the reports revealing significantly higher levels in OSCC patients, utilization of inflammatory cytokine proteins such as IL-8 and IL-1β as biomarkers has been debated [43]. Considering the wide range of complex events these proteins regulate following various stimuli including mechanical, bacterial, chemical and environmental stress, the actual behavior patterns of IL-8 and IL-1β in oral carcinogenesis require thorough evaluation [44]. Therefore, we suggest that an immunological/inflammatory disease control group should be included in future studies which investigate the actual efficacy of establishing the salivary IL-8 and IL-1β and other markers, as diagnostic markers for OSCC. To the authors knowledge, the salivary IL-8 levels in patients with severe periodontal disease were also evaluated in only one case-control study, and patients with oral cancer presented significantly higher saliva levels of IL-8 and IL-1β, indicating that presence of an immunological disease does not increase these cytokines levels as high as OSCC [14]. Ten periodontitis patients were enrolled in that particular study and this assumption was concluded in a very limited periodontitis patients group. Considering the literature related to the wide range of cytokine levels especially IL-8 and IL-1β both in healthy, OSCC and periodontitis patients, there is little doubt that larger sample size controlled studies may contribute further scientific data to this issue.

In future studies, meticulous dental, periodontal and radiological examinations should be performed in order to establish the oral inflammatory/infection status of study subjects, since these potentially confounding conditions may influence the accuracy of salivary biomarkers in early detection of OPMDs and OSCC lesions. The goal should be continuing development of salivary and other detection/diagnostic adjuncts to improve detection, prognosis and guide in management and outcome prediction of OPMDs and OSCC.

Conflict of interest

The authors declare that they have no conflicts of interest.

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