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Letter to the Editor

Comment on "Simultaneous quantification of metronidazole, tinidazole, ornidazole and morinidazole in human saliva": Saliva or gingival crevicular fluid?

In a recent paper, Wang et al. [1] reported the simultaneous quantification of metronidazole, tinidazole, ornidazole and morinidazole in human saliva. The authors developed and validated a rapid and sensitive method, HPLC-UV detection, for the simultaneous quantification of metronidazole (MEZ), tinidazole (TNZ), ornidazole (ONZ) and morinidazole (MNZ) in human saliva, using one mobile phase. The study seems attractive and interesting, however, there are some issues about which we feel doubtful and would like to discuss with the authors and other readers.

The first doubt is about the samples collected from the subjects. The samples of the research seem to be confusing in Wang et al.'s article. In the abstract, Wang et al. claimed the aim of the study was to develop a rapid and sensitive method for the simultaneous quantification of four drugs in human saliva, as their article entitled 'simultaneous quantification of metronidazole, tinidazole, ornidazole and morinidazole in human saliva'. Based on that, the main object of their research was supposed to be "saliva". Moreover, only saliva sample, instead of GCF, was mentioned in the sample preparation and results. However, in the discussion and conclusions, Wang et al. described that 'the concentration of MNZ and ONZ found in crevice fluid after a 50-min i.v. infusion reach a saliva level of more than 4100 ng/ml' and then drew the conclusion that their method 'was successfully applied to the analysis of clinical samples obtained from saliva and gingival crevicular fluid (GCF) in Phase II clinical trials of MNZ'. Here we are confused by how they drew this conclusion about GCF.

Second, the position of collecting saliva samples also made us puzzled. As described in the paper, Wang et al. claim that they collected saliva and GCF 'from the gingival crevice fluid samples from patients with periodontal diseases by way of aspiration through a capillary micropipette into 1.5-ml tubes'. However, we suspect that the samples collected from the subjects could be GCF rather than saliva. Although saliva and GCF are oral secretions, they come from different sources with different composition. In fact, the whole saliva is a mixture of oral fluids from the major salivary (submandibular 65%, parotid 23% and sublingual 4%), minor salivary glands (8%), and nonsalivary origin including GCF, serum transudate from the mucosa and sites of inflammation, epithelial and immune cells, food debris and many microbes [2]. Accordingly, GCF is not a substitute for saliva, rather, it is only a small part of the whole saliva. The constituents of GCF originate from serum, gingival tissues, and from both bacterial and host response cells present in the aforementioned spaces and the surrounding tissues. GCF can be found in the physiologic space (gingival sulcus), as well as in the pathological space (gingival pocket or periodontal pocket) between the gums and teeth [3]. It shows that GCF is different from saliva not only in origin but also in composition. Only GCF, rather than saliva, can be collected from gingival crevice. Based on that, we assume that the position Wang et al. collected saliva samples is wrong and that by using this method it is not possible to collect saliva from the site Wang et al. mentioned.

If the subject of the research was saliva as the authors expressed, different collection methods were supposed to be used in the research. To date, many methods of collecting saliva samples are discussed, and it can be collected with or without stimulation. For example, Kumar et al. [4] collected about 2 ml of saliva over 5–10 min in a universal container. To facilitate salivary secretion, the individuals were asked to chew a piece of unsweetened, unflavored chewing gum and spit out the initial salivary secretion. Orti et al. [5] collected parotid saliva samples by means of a modified double-lumen parotid cup. Orange-flavored lozenges served as a reflex stimulus to induce salivation. In our study [6], human saliva samples was absorbed on Whatman filter paper strips (2 mm × 8 mm), contained in 1.5 ml Eppendorf tubes. The filter paper strips were left on the tongue for 30 s. They were weighed before and after application on analytical balance, and then 200 μl PBS aside was added to the tube, standing for 1 h, stored at −20 °C. The saliva samples were naturally secreted by healthy adults, thus avoiding the influence of age, chemical irritants, and other factors on the subjects.

Second, suppose the main subject of the study was GCF, the testing of linearity might be under discussion. According to some research [7,8], the common way of collecting GCF is through filter paper, though capillary method is also considered as one of sampling methods. GCF is a serum transudate or inflammatory exudates. As such, the fluid reflects the constituents of serum, the cellular response in the periodontium, and contributions from the gingival crevice [9]. GCF reflects the condition of the gingiva and contains proteins transuded from serum or cells at inflamed sites [10]. Compared with saliva, the drug concentration in the GCF in periodontitis treatment may be of more therapeutic significance. Considering that GCF samples are usually less readily available, we suggest that the calibration curves of GCF testing may use serum instead or utilize Bastos' method [7]. We notice that no test results of GCF are mentioned in the article and we wonder the reason for that.

Combining all of the above aspects, we propose that the main samples of Wang's experiment might not be saliva as the authors described; rather, they are GCF. In addition, the results of the study are not in accordance with the discussion. Which one should be the sample of the study, saliva or GCF? The question is expected to be answered by more discussions and considerations.

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