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In reference to Analysis of reflux as the aetiology of laryngeal dysplasia progression through a matched case-control study.

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October 5, 2020

Dear Editor,

We reviewed the article entitled: "*Analysis of reflux as the etiology of laryngeal dysplasia progression through a matched case-control study* ".[1] The authors did not find differences in the level of pepsin, enterokinase and bilirubin in laryngeal dysplasia (LD) of patients with malignant transformation*versus* those without transformation. The involvement of reflux in the development of LD and laryngeal cancers is an important topic and the realization of such a study is important. However, we wish to draw attention to many points.

First, it is difficult to know if the included patients with tissue pepsin really suffered from reflux. The detection of pepsin into the tissue means that patients had some pharyngeal reflux events the day before the surgery but cannot confirm the diagnosis. The sensitivity of pepsin detection in laryngeal tissue depends on the technique and the material (antibodies), reaching 75 to 85% depending on the type of reflux (acid *versus* nonacid).[2] Moreover, we have no detailed information about the immunostaining technique, limiting the reproducibility of the protocol. The presence of pepsin into the tissue does not ensure the reflux diagnosis. Thus, for example, it has been showed that the back flow of gastric content and the deposit of pepsin into the tissue are influenced by the meals preceding the sample collection, making the pepsin tissue a poorly reliable marker of reflux.[3] To improve the sensitivity, authors[1] could have performed hypopharyngeal-esophageal pH-impedance monitoring, which is the only way to confirm the diagnosis.[4]

Second, the LD malignant transformation involves many factors such as tobacco history, environmental factors, genetic, or immune response.[5] The authors did not provide information about the tobacco history (pack-year data) of groups, which is an important data to consider the risk of malignant transformation. Even many years after the tobacco cessation, it is conceivable that patients with long/more severe history of tobacco consumption may have more cell mucosa DNA impairments and a higher risk to develop cancer.

Third, the focus on pepsin as the only enzyme associated with malignant transformation limits the understanding of transformation mechanisms. More than 50% of patients had mixed or nonacid reflux,[4] in which the activity of pepsin is decreased regarding the alkaline pH of refluxate. To reliably investigate the involvement of reflux in the malignant transformation, authors have to consider the entire content of refluxate, including bile salts and trypsin.[4] Furthermore, bile salts may be involved in laryngopharyngeal malignant transformation.[6]

In future studies, reflux has to be diagnosed at the LD diagnosis time and physicians have to follow the reflux clinical course over the time. More than 50% of reflux patients had chronic course,[4]which leads to a potential higher risk to develop cell DNA damage and lesions. Thus, cross-sectional study design is probably not adequate to study a disease association involving chronic and repeated exposure.

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Acknowledgments: No.

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