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Letter to the Editor

Comments on “The Prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* in Relation to Periodontal Disease among Patients Attending Dental Clinics in Urmia, Iran: A Cross-Sectional Study”

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Dear Editor-in-Chief

We read with interest the recent article by Azadeh et al (1). The authors should be commended for investigating the prevalence of oral protozoa using both microscopic and molecular methods. Nevertheless, several methodological and analytical issues merit clarification because they may influence the interpretation of the findings.

Our primary concern relates to the inconsistency between the study objective and the study design. Both the title and the stated objective imply an evaluation of the relationship between *Entamoeba gingivalis*, *Trichomonas tenax*,

and periodontal disease (2). However, all 133 enrolled participants had chronic periodontitis, and neither healthy individuals nor patients with gingivitis were included. Consequently, the study cannot determine whether these protozoa are associated with periodontal disease; rather, it estimates their prevalence among patients with established periodontitis. Therefore, statements suggesting a role of these protozoa in periodontal disease should be interpreted with appropriate caution.

The statistical analysis also deserves further consideration. Several established risk factors for periodontal disease, including age, sex, smoking, diabetes, cardiovascular disease, oral hygiene, and antibiotic use, were evaluated only



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by univariate analyses. Since these variables may confound one another, adjusted analyses using multivariable regression would have provided more reliable estimates of independent associations, as recommended by the STROBE Statement for observational studies (3). In addition, odds ratios were presented without corresponding 95% confidence intervals, making it difficult to evaluate the precision of the reported estimates.

We also noted an apparent inconsistency in the statistical results. In Table 5 of their paper, the association between sex and *T. tenax* infection is statistically significant ($P = 0.037$), with an odds ratio of 2.1. However, the Discussion states that this association was not statistically significant ($P = 0.063$). Moreover, the crude frequencies presented in that table do not appear to correspond to the reported odds ratio. Because consistency between tabulated data and textual interpretation is essential for accurate scientific reporting, clarification of these discrepancies would be valuable.

Furthermore, statistical analyses were performed for variables with extremely small sample sizes, including asthma ($n = 1$), pulmonary disease ($n = 3$), and household purified water users ($n = 3$). Such sparse data can produce unstable effect estimates and unreliable significance testing and therefore should be interpreted cautiously.

Finally, although PCR detected substantially more positive samples than direct microscopy, the statement that molecular methods demonstrated "increased sensitivity" may overstate the available evidence (4). Diagnostic sensitivity can only be established by comparison with an appropriate reference standard or by formal diagnostic accuracy analyses. Similarly, because of the cross-sectional design, the conclusion that *E. gingivalis* may play a greater role in periodontal disease pathogenesis should be

regarded as hypothesis-generating rather than causal.

We hope these comments will contribute to a balanced interpretation of the findings and encourage further well-designed studies to clarify the epidemiological and clinical significance of oral protozoa in periodontal diseases.

Conflict of Interest

The authors declare no conflict of interest.

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