Feasibility Of Molecular Diagnosis Of *Entamoeba Gingivalis* In Periodontal Disease

Thesis

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Summary

It was documented that *Entamoeba gingivalis* is the first commensal protozoa found in the human oral cavity, and it is usually exists in gingival tissues, particularly in suppurative and inflammatory conditions. This is due to its preference for anaerobic environments. Some studies found high incidence of *E. gingivalis* in patients with periodontal disease. This suggests that this protozoan might play an important role in the etiology of the periodontal disease. However, because it is also present in the oral cavity of healthy subjects, some authors suggest that this commensal protozoan could be opportunistic, that is, it can proliferate in a gingival tissue modified by periodontal disease.

The main aim of the present work was to assess the feasibility of isothermal molecular technique in the diagnosis of *Entamoeba gingivalis* in patients with chronic periodontitis in comparison to traditionally used microscopy aided by some staining methods.

Objectives of the study:

1- Compare between the traditionally used microscopic method for diagnosing this protozoan infection and a relatively simple molecular technique.
2- To assess the prevalence of *Entamoeba gingivalis* infection in periodontal diseases.
3- Investigate some risk factors related to *Entamoeba gingivalis* infection.

This study included (50 subjects) suffering from gingivitis or/and chronic periodontitis (group 1) and (50 subjects) healthy volunteers (group 2). These cases were diagnosed according to their clinical examination. Data

sheet and dental plaque samples were collected from each subject, dental plaque samples were stained (in tripartite) with Trichrome stain and H&E stain and subjected to microscopic parasitological examination then a molecular diagnosis was done for all samples.

Microscopically, the present study showed that the cytoplasm of *E. gingivalis* trophozoite appears light pink in color with darker colour nucleus by H&E stain. In Trichrome stain, the cytoplasm of the trophozoites was blue-green tinged with purple while the nuclei and inclusions appeared purple-red in colour. Leuko-phagocytosis was observed in many *E. gingivalis* trophozoites which denote engulfment of white blood cells (WBC's) by the detected trophozoites.

Isothermal LAMP run was done twice after initial hot start technology. Positive products were visualized by different means; increase in turbidity of the mixture while the negative sample remained clear. In addition; direct visual check after addition of SYBR Green I revealed that positive reactions changed to green. Then, under UV illumination, positive reactions turned bright green while negative reactions remained uncolored. Agarose gel electrophoresis of positive samples was done and revealed a ladder of multiple bands while no bands were detected in the negative control.

The prevalence of *E. gingivalis* in the diseased cases and control subjects was 41(41%) samples out of the total 100 collected samples. 31 of them were from group of cases complained of gingivitis and C. P. and the remaining 10 subjects were from the control group. The higher

occurrence of the parasite within the diseased group was statistically significant ($P \leq 0.05$).

By using H&E stain, 13 samples related to the diseased cases (26%) and 7 (14%) related to the control healthy group were confirmed microscopically positive. While performing trichrome stain, positive samples were reported among 7 samples from the diseased cases (14%), but this stain didn't detect any subject in the control group. By LAMP, 31 belonging to the diseased cases (62%) and 10 subjects (20%) in the control group obtained positive finding in the 2 LAMP runs.

Isothermal PCR with hot start gave the highest sensitivity 100% and highest specificity 100% while H&E gave 48.8% sensitivity and 100% specificity. Trichrome stain gave the lowest sensitivity and specificity, 17.1% and 100% respectively.

The impact of smoking and the effect of hygiene among positive and negative groups were statistically significant ($P \leq 0.05$). The presence of WBC's, fungus and bacteria among the negative and positive groups were significantly observed in a higher number of positive samples for *E. gingivalis* more than those of negative findings ($P \leq 0.05$). WBC's were as well significantly higher in *E. gingivalis* positive subjects compared to negative subjects within the diseased group.

There were a higher number of *Entamoeba gingivalis* positive controls with bad oral hygiene than those of *E. gingivalis* negative controls. Again WBC's were significantly higher in *E. gingivalis* positive subjects compared to the negative subjects within the control group.