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Short Communication

The Oral Parasitic Microbiome in Hepatitis B Virus Infected Sudanese Patients with Gum Disease

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Abstract

Background: We aimed to evaluate a potential link between colonization of gingival crevices by the *Entamoeba gingivalis* as oral parasite microbiome and Hepatitis B infection among gum disease Sudanese patients.

Methods: This study was conducted on 448 gum disease patients attending to Dental Clinic of Kosti Teaching Hospital, Kosti, Sudan in 2017-2018. Oral symptoms were registered in 336 patients at different stages of the HBV infection and in 112 HBV seronegative gum disease control. All participants were screened for HBV using ELISA test. Unstimulated whole saliva and gingival scraping were obtained and analyzed for the presence of the amoeba using a parasitological technique.

Results: Statistically highly significant correlation was found between the detection of the *E. gingivalis* in Saliva/gingival scraping and gum illness disease with HBV-infected patients than healthy control group ($P<0.05$). There was high association between the occurrence of the amoeba among the two groups and smoking, snuffing habitats ($P<0.05$), inversely, no association with the oral personal hygiene.

Conclusion: The presence of the amoeba was not related to the degree of gum diseases only, but to the HBV infection diagnosis. To our knowledge, this is the first study of *E. gingivalis* in association with HBV infection among gum disease Sudanese patients; maybe predict the role of oral parasitic microbiome in the status of gum disease in HBV infection.

Introduction

A gum disease is a public health issue, being one of the most prevalent diseases worldwide. It is the second most common oral lesion after oral candidiasis (1). The main stages of gum disease are gingivitis and periodontitis. Usually gingivitis precedes periodontitis. Periodontal disease may progress painlessly, producing few clear signs, even in the late stages of the disease.

Hepatitis B is a life-threatening illness and hepatic cirrhosis and hepatocellular carcinoma can be developed in asymptomatic carrier (2-5). Hepatitis B is hepatotropic virus disease, still a major health problem despite the availability of vaccination, and is a result of a host immune response and the infection is notcytopathic (6,7). The causes of gum oral lesions and recurrent ulcerations among patients infected with HBV are still unknown (8, 9). The risk for progressive extensive necrosis has been described, and the condition should, therefore, be carefully diagnosed, treated, and followed up (10). Healing of oral lesions have been difficult to achieve with a tropical treatment of a viral infection that causes host immunodeficiency (10), but uses of metronidazole has been reported to be clinically effective (8).

Entamoeba gingivalis is a protozoan parasite that belongs to the genus *Entamoeba* that inhabits the oral cavities. It is thought to be a commensally oral microbiome in humans. *E. gingivalis* amoebae was detected early in the mouth as the first symbiotic microbiome described in humans (11). It is an oral microbiome found only in its trophozoite form (12). It may be present on the teeth and gingival surface, lesions, interdentally spaces, and in pockets of gingival (13, 14). Additionally, it has been known to inhabit the bronchial mucus and tonsillar crypts. *E. gingivalis* can be found in 95% of people with gum disease and 50% with healthy gums as hosts. The transmission occurs directly from one person to another through kissing, sharing eating utensils, or via droplet spray.

E. gingivalis pathogenicity in the oral cavity is still not completely understood. It is presence in the patients with a good state of immunity usually does not cause pathological effects or changes (13, 15). Age, dental caries, and oral hygiene are considered important factors, which play a role in the *E. gingivalis* pathogenicity (14). Furthermore, the occurrence of this parasite is correlated and associated with the age of host (16). Metabolic disabilities favor gum tissues pathological changes, therefore, may influences types or species composition of protozoan, virus and or bacterial infections in patients with different systemic illnesses (17).

Screening for oral parasitic microbiome in saliva and gingival scraping has shown significant changes in the viral infection. It is rarely studied in developing countries (18). Valuable knowledge will be missed, if this parasite not screened or studied in HBV infected patients with gum disease to understand the mechanism or the role of the disease and improve public health in developing countries, this study was carried out to detect and identify *E. gingivalis* as an oral parasitic microbiome in whole saliva and gingival scraping specimens and to relate the prevalence of the parasite and HBV infection among gum diseases Sudanese patients.

Materials and Methods

Patients and Sample Collection

A hospital-based study was conducted on four hundred forty-eight gum disease patients attending to Dental Clinic of Kosti Teaching Hospital, Kosti, Sudan in 2017-2018. Overall, 336 of the patients were HBV infected patients group. Moreover, 112 cases were non-

infected HBV patients as a control group. None had removable dentures. Serum, whole saliva and gingival scraping specimens were analyzed for all patients. None of all patients were receiving any medication. Smoking and snuffing habits as well as oral hygiene were registered.

Serum Sample Collection and HBV screening

Five ml of a blood sample was collected from each patient, centrifugated (500g x 5 min), and the obtained serum was used for HBV screening test, using a commercial ELISA diagnostic kit for HBsAg (Shenyang Huimin Biotechnology Co. Ltd, Shenyang, China), and followed the manufacturer's instructions.

Saliva Sample collection and detection of the amoeba

Unstimulated whole Saliva samples were collected (19), from patients attending the dental clinic of Kosti Teaching Hospital, Kosti, Sudan. Saliva was placed in Sodium acetate formalin (SAF) fixative for analysis of *E. gingivalis*. Saliva fixed samples were centrifuged (500g x 5 min). The slide preparation stained by trichrome stain (20), and every slide scanned for an average of 5-15 min using one hundred oil immersion fields of the light microscope.

Gingival scraping Samples collection and detection of the amoeba

Gingival Scraping samples obtained by swab or spatula, placed in a clean, airtight container and then extracted from swab or transferred from the container for examination (21). The scrapped samples were prepared in a clean slide and fixed by absolute methanol, stained by Trichrome stain and one hundred oil immersion fields of light microscope used in an average of 5 -15 minutes (19,20).

Statistical analysis

The differences between groups of subjects were analyzed by Fisher's exact test, Student's t-test, and Chi-square using Windows software for Social Sciences (SPSS 21.0) (Chicago, IL, USA). Data were considered to be statistically significant when the probability of type I error was 0.05 or less.

Ethical Approval

The patients were aware that their serum, unstimulated whole saliva and gingival scraping samples were needed for the diagnosis of the diseases, in the study using serological and parasitological methods. The research approved by the hospital Ethics Committee of Kosti Teaching Hospital, Kosti, Sudan, in accordance with the Helsinki Declaration and guidelines.

Results

A highly significant correlation was found between the detection of the *E. gingivalis* in Saliva/gingival scraping and gum illness disease in the HBV-infected and non-infected patients and the prevalence of *E. gingivalis* was higher among HBV-infected patients had been 178 males and 158 were females (227 of 336; 67.6%) than in non-infected HBV patients had been 59 males and 53 females (36 of 112; 32.1%), therefore, *E. gingivalis* was more common in gum disease patients infected with HBV, and the severity of gum disease increased in the HBV-infected patient more than control group.

The detection of *E. gingivalis* statistically significant between Saliva and gingival scraping in the non-infected HBV patients group. There also was a significant difference between the saliva sample and gingival scraping in the detection of *E. gingivalis* in the HBV infected group as shown in (Table 1).

The high association between the occurrence of the amoeba between the two groups and smoking habitat, and also there was a high significance between the presence of *E. gingivalis* and snuffing habitat among the two groups as shown in (Table 2). There was no association between oral hygiene and the pres-

ence of *E. gingivalis* in the two groups and the distribution of *E. gingivalis* in non-infected HBV patients group and HBV infected group was (23 of 112; 69 of 336 with bad hygiene) and (89 of 112; 267 of 336 with good hygiene), respectively.

Table 1: The occurrence of *Entamoeba gingivalis* detected in gingival scraping and saliva of gum disease in between the two groups

Occurrence	*non-infected HBV group (n=112)*	*Mean*	*SD*	*P-value*	*HBV infected group(n=336)*	*Mean*	*SD*	*P-value*
On Saliva	36	1.67	.469	<0.001	227	1.32	.469	<0.001
On gingival scraping	28	1.75	.435		198	1.41	.493	

Table 2: *Entamoeba gingivalis* in non-infected HBV patients and HBV infected groups between smoking and suffering habitats

Occurrence	*Non-infected HBV patients group(n=112)*	*HBV group(n=336)*	*P-value*
Smoker	45	64	<0.001
Non-smoker	67	272	
Snuffer	28	50	0.003
Non snuffer	84	286	

Discussion

E. gingivalis is a protozoan parasite, which belongs to the genus *Entamoeba* that inhabits the oropharynx and is thought to be a commensally oral microbiome in humans (21-23), it is commonly found in cytological and histopathology specimens, and more common in patients with poor oral hygiene, bad dentition, or with immune suppression. However, the identification of oral parasites microbiome rarely studied in developing countries, especially in the relation of *E. gingivalis* with infectious diseases such as HBV infection. Useful information's will be lost if the *E. gingivalis* not screened and detected in HBV infected patients, therefore, improve the patient health status and prognosis of the disease. Besides, HBV infection other predisposing factors such as life stress, inadequate sleep and oral bad hygiene are factors that increase the severity of gum diseases (9, 24, 25).

Our present work showed a high significant difference in the detection of *E. gingivalis* between Saliva and gingival scraping in the infected and non-infected HBV patients, with a high increase in detection of the amoeba among Saliva samples than gingival scraping. Saliva environment condition may be more suitable for the amoeba growth and movement than gingival tissue. Metabolic disabilities favor gum tissues pathological changes, therefore, may influences the amoeba in HBV infection (7, 15, 17). *E. gingivalis* is an opportunistic organism that harbored an immunocompromised host. Hepatitis b virus infection is cell-mediated immune disease result from a host immune response and not cytopathic (1, 6,7).

Our study for the first time expresses the association of gum diseases with HBV infection in Sudanese dental patients and no similar studies. *E. gingivalis* has been identified in present investigation, from obtained gingival

scraping and Saliva specimens using the light microscopy, which is helpful and effective in developing countries for, identify *E. gingivalis* in diseased gingival pockets (11,21).

The role of laboratory diagnostic uniqueness is important and may have therapeutic implications: while *E. gingivalis* is a metronidazole susceptible parasite and metronidazole is clinically effective and safe in the treatment of orofacial parasitic infection (26). Further studies should be done to evaluate the effectiveness of metronidazole in gum illness patients infected with HBV.

Conclusion

There was a clear difference in the present detection and identification of *E. gingivalis* as an oral parasitic microbiome in gum diseases infected with HBV infection and non-infected HBV patients. Additionally, saliva is a sample of the best choice for the detection and identification of *E. gingivalis* in a clinical laboratory. To our knowledge, this is the first study of *E. gingivalis* in association with HBV infection among gum disease Sudanese patients; and maybe predicts patients' health status and may explain the role of the oral parasitic microbiome in the degree of gum disease in HBV infection.

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Conflict of interest

The authors declare that there is no conflict of interest.

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