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Study of immunological parameter (ECP) of entamoeba gingivalis in immunocompromised patients with periodontitis in Al-Najaf Al-Ashraf City

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Abstract---Objectives: The present study aimed to compare the presence of *E. gingivalis* in immunocompromised patients with periodontitis and control group by measuring immunological parameter (ECP) by using ELISA. Methodology: A Comparison case-control study design was done on 80 samples [40 case (20 immunocompromised patients with periodontitis + 20 immunocompromised patients without periodontitis) were selected from Al-Sader Medical City/Middle Euphrates Cancer Center and 40 control (Diabetes Mellitus patients with poor oral sanitation)] were selected from Al-Najaf Center for Diabetes and Endocrinology/Al-Sadr Medical City, during the period from the beginning of November 2021 to the end of February 2022. Age range of cases and control between (20 - ≥ 60) years. Results: The results of the Salivary Tests (Immunological Cytokines Assays) showed the concentration levels of ECP was (6.6860 ± 2.97386Pg/ml) in immunocompromised patients with Periodontitis. But in immunocompromised patients without Periodontitis was (4.7280 ± 3.46401 Pg/ml). While in control group (Diabetic Mellitus Poor Oral Sanitation) was (49.294 ± 22.42156 Pg/ml). Conclusion: The infection rate of the *E.gingivalis* parasite in the control group (Diabetic Mellitus Poor Oral Sanitation) was twice higher than that of the immunocompromised patients with periodontitis and without periodontitis. ECP highly increase in Control group infected with *E.gingivalis* and highly decreased in immunocompromised with Periodontitis and immunocompromised without Periodontitis respectively.

Keywords--Entamoeba gingivalis, immunocompromised, periodontitis, eosinophil cationic protein.

Introduction

Many people suffer from infection with *Entamoeba gingivalis* which mainly causes for periodontitis and other dental diseases specially in patient suffering from Immunity Issues. Periodontitis was identified by irritation, halitosis, discomfort, hemorrhage and loss of healthy gum or teeth. It is estimated that 5% - 20% of the world population suffer from at least one oral cavities disease (Punna-Moorthy, 2019). Several factors have been identified as the pathogenic agents for diseases including viruses, bacteria, fungi and parasites. The possible role of oral protozoa has been investigated yielding controversial results. Two common oral cavity protozoa are *Entamoeba gingivalis* and *Trichomonas tenax*, whose role in causing oral and dental diseases (Eke *et al.*, 2020).

E. gingivalis contamination was reported in oropharynx as the commensal scavenger organism and in patients with poor oral sanitation, immunocompromised patients and diabetic patients .The infection is developed by the transmission of the trophozoite through oral connection The parasite is also found in saliva (García *et al.*, 2018). Immunocompromised patients people who have weakened immune system and reduced ability to fight infections and other diseases. This may be caused by certain diseases or conditions, such as AIDS, cancer, diabetes, malnutrition, and certain genetic disorders. It may also be caused by certain medicines or treatments, such as anticancer drugs, radiation therapy, and stem cell or organ transplant (Azoulay *et al.*, 2020).

Eosinophil Cationic Protein (ECP) is a basic protein present in the granules of the eosinophil in different forms, playing a role in the host's immune response to parasites and this major basic protein usually toxic to parasites (Kaneva *et al.*, 2020). The present study aimed To determine the prevalence of the *Entamoeba gingivalis* among immunocompromised patients and to evaluate the status of Eosinophil Cationic Protein (ECP) in saliva of immunocompromised patients with periodontitis.

Methodology

Patients Group

Samples collection (40 Oral swap and 40 Saliva specimens) were conducted for immunocompromised patients with periodontitis (20 participant) and immunocompromised patients without periodontitis (20 participant) during the period from the beginning of November 2021 to the end of February 2022 at Al-Sader Medical City/Middle Euphrates Cancer Center and their ages ranged between (20 - ≥ 60) years, and a direct questionnaire was conducted with all the patients participating in this study are as described in Appendix 1.

Control Group

A group of volunteers (diabetic patients with poor oral sanitation) who they were selected to participate in this study as the control group from AL-Najaf Center for Diabetes and Endocrinology / Al-Sadr Medical City. The control group was matched with the patient group in terms of age and sex. For the control group (40 participant) during the period from the beginning of November 2021 to the end of February 2022 and their ages ranged between (20 - ≥ 60) years.

Samples Collection (Oral swap and Saliva)

Oral swap for mouth is directly done using a sterile cotton swab and transferred to the parasitology department. The saliva collecting from same patients in tube and transferred to the freezer and frozen in -20 C° for immunological examination by ELISA.

Study Location

This case control study was conducted in Al-Najaf city of Iraq.

Excluded Criteria

Comprised consuming systemic antibiotics in the past two months, using immunosuppressive drugs, smoking, being pregnant and having systemic, heart or respiratory diseases.

Microscopic Examination

Oral swap for mouth is directly done using a sterile cotton swab and the sample is placed on a glass slide, a drop of normal saline solution is placed on the sample, and the sample is examined under a light microscope under the forces of (10x) , (40x). The positive result can be observed by seeing the parasite movement characteristic of it, where the mouth parasites move in a spiral manner.

ELISA Technique

Estimation concentration level of Eosinophil Cationic Protein (ECP) done in lab according to Mybiosource/USA protocol.

Statistical Methods

The following Statistical analysis approach by using social sciences (SPSS) version 20 in order to analyzed and assess the data of the study, t test and LSD was applied to find out the significant difference between the data. Differences were recorded as significant whenever the probability (P) was less than 0.05.

Results and Discussion

Isolation and Identification of *Entamoeba gingivalis* in immunocompromised Patients with and Without Periodontitis and Control Subjects

Out of (80) oral swap samples that which examined microscopically for *E.gingivalis* by using light microscope as shown in Figure (1) under (40x), *E.gingivalis* infection detected in 11 (29.7%) out of 20 immunocompromised Patients with Periodontitis, and in 3 (8.1%) out of 20 immunocompromised Patients without Periodontitis. While in the control subjects, 23 (62.2%) out of 40 DM Poor Oral Sanitation were positive for *E.gingivalis* infection with statistically significant (P Value = 0.005) as shown in Table (1).

Many pathogenic agents as well as systemic and immunosuppressive diseases can cause major periodontal problems, among the current protozoa of the oral cavity, *E. gingivalis* is the most frequent parasite. The role of this parasite in inducing periodontal diseases is not exactly clear and remains controversial. To define the pathogenic role of the parasite, appropriate treatment schedules should be set up by dentists (Rahdar *et al.*, 2019).

The total percentage of *E.gingivalis* in the presented study was 46.25% this nearly similar to Al-Dulaimi *et al.*, 2020 where found *Entamoeba gingivalis* in Babylon Province was (42.9 %). The results of the current study was much higher than what Rahdar found in his study that conducted in Iran, reported that only 16% of the patients were infected with *E. gingivalis*. The study presented by Ghabanchi *et al.*, 2010 found the prevalence of oral *E.gingivalis* in southern Iran was (66.7%), while Sharifi *et al.*, 2020 found the prevalence of *E. gingivalis* was 11.7% also in Southeastern Iran, and this results that reported by Sharifi was much lower than what we found in our study.

The results of our study found statistically significant (P Value = 0.005) between study groups but this uncompatible with (Rahdar *et al.*, 2019) who found no significant difference between the study groups (P > 0.05), and said the presence of *E. gingivalis* cannot induce periodontal lesions and other factors are involved. The study presented by Ghabanchi *et al.*, 2010 found the prevalence of oral *E.gingivalis* in southern Iran was (66.7%), while Sharifi *et al.*, 2020 found the prevalence of *E. gingivalis* was 11.7% also in Southeastern Iran, and this results that reported by Sharifi was much lower than what we found in our study.

The study presented by Badri revealed a high prevalence rate of the infection among periodontal disease patients with 37% (95% CI 20-57%). To conclude, it must be considered that *E. gingivalis* can be a risk factor associated with oral diseases and a wide range of research is needed to specify its role in the pathogenesis of these disorders (Badri *et al.*, 2021).

Table (1): Distribution of respondents according to microscopically diagnosis

Microscopically Diagnosis			GROUPS			Total	p-value		
			With Periodontitis	Without Periodontitis	DM Poor Oral Sanitation		F	df	p-v
	No.	Count	9	17	17	43	10.509	2	0.005
		% within	20.9%	39.5%	39.5%	100.0%			
Entamoeba gingivalis		Count	11	3	23	37			
		% within	29.7%	8.1%	62.2%	100.0%			
Total		Count	20	20	40	80			
		% within	25.0%	25.0%	50.0%	100.0%			

Salivary Tests (Immunological Cytokine Assay OF ECP) for The Studied Groups

Table (2) shows the results of the salivary tests (Immunological Cytokines Assays) the concentration levels of ECP was (6.6860 ± 2.97386 Pg/ml) in immunocompromised patients with Periodontitis. But in immunocompromised patients without Periodontitis was (4.7280 ± 3.46401 Pg/ml). While in control group (Diabetic Mellitus Poor Oral Sanitation) was (49.294 ± 22.42156 Pg/ml). There are no published studies regarding ECP with immunocompromised patients with periodontitis suffered from E. Gingivalis infection and nor with Diabetic Mellitus.

But Ribas *et al.*, 2018 was found that neutrophils from patients with periodontitis express higher levels of ECP than those from healthy volunteers. This contradicts the results of our current study.

Table (2): Eosinophil Cationic Factor Concentration in Studies Groups of immunocompromised patients with Periodontitis, Without Periodontitis and control subjects (DM Poor Oral Sanitationand)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ECP	With Periodontitis	20	6.6860	2.97386	.66497	5.2942	8.0778	1.60	11.61
	Without Periodontitis	20	4.7280	3.46401	.77458	3.1068	6.3492	1.61	13.03
	DM Poor Oral Sanitation	40	49.294	22.42156	3.54516	42.1235	56.4650	10.11	90.16
	Total	80	27.500	27.10450	3.03038	21.4688	33.5324	1.60	90.16



Figure (1): *Entamoeba gingivalis* under Light Microscope (The arrows→ refer to trophozoite of parasite 40X)

Conclusions

1. The infection rate of the *E.gingivalis* parasite in the control group (Diabetic Mellitus Poor Oral Sanitation) was twice higher than that of the immunocompromised patients with peridontitis and without peridontitis.
2. ECP highly increase in Control group infected with *E.gingivalis* while highly decreased in immunocompromised with Periodontitis and immunocompromised without Periodontitis respectively

Recommendations

The present study recommends the following points:

1. Molecular diagnostic study for detection sub species of *E.gingivalis* in patients with mouth ulcers.
2. Immunological study to compare the level of ECP concentration in patients with type 1 and type 2 diabetes mellitus.

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Ethical Standards

The current study obtained ethical approval by the Department of Medical Laboratories in the College of Health and Medical Technologies / Kufa, Najaf Health Department / Training and Development Center, and written consent was taken from all participants in the research (patient group and control group).

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