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Prevalence of Periodontal Pathogens in a Group of Egyptians with Emphasis on Correlation between Periodontal Stages and Molecular Bacterial Identification.

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ABSTRACT

Purpose: This study was performed for identification and prevalence of periodontal microbiota in a group of Egyptians with a special emphasis on the correlation between different periodontal stages and molecular bacterial identification. **Subjects and methods**: 100 Subjects were divided equally into five groups with reference to new classification of periodontitis. Clinical parameters including probing pocket depth (PPD), clinical attachment level (CAL) gingival index (GI) and plaque index (PI) were measured. Samples of subgingival plaque were taken for microbiological analysis and bacterial DNA extraction. Bacterial identification was done using polymerase chain reaction (PCR) for 5 bacteria (Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Treponemadenticola (Td), Tannerellaforsythia (Tf), Fusobacterium nucleatum (Fn) in all samples for the study population. **Results:** The mean plaque index, gingival index, PD, and CAL were calculated, and the prevalence of P.g, T.d, T. f,A.a and F.n was also identified. **Conclusion:** for all kinds of bacteria the mean of real time increased by increasing the stage number. The difference between the healthy group and all stages was statistically significant. We can arrange the bacteria sequence as follows based on the overall mean of real time PCR for each type of bacteria: T.f,P.g, T.d,F.n,A.a. It was also clear that the relationship between PD and CAL and other bacterial microbiota was statistically significant.

KEYWORDS

Periodontal diseases, PCR, Egyptians.

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INTRODUCTION

Periodontitis is a bacterial infection-induced inflammatory immune-mediated disease that causes the deterioration of the tissues around the tooth [1]. Epidemiological data from countries around the world indicate a consistent pattern, showing that roughly 10% of all communities, adults over the age of 40–50 will develop severe periodontitis, with the danger of losing teeth during their lifetime [2].

The new periodontitis classification framework uses a multidimensional staging and grading system that can be changed when new information is available [3] Staging is largely determined by the severity of the disease at presentation and the complexity of disease management, whereas grading provides additional information about biological features of the disease, such as a history-based analysis of the rate of disease progression, assessment of the risk of further progression, expected poor treatment outcomes, and assessment of the risk that the disease or its treatment will have a negative impact on the patient's overall well-being [3,4].

Staging is divided into four groups (stages 1 through 4) based on a number of factors such as clinical attachment loss, bone loss amount and percentage, probing depth, presence and extent of angular bony defects and furcation involvement, tooth mobility, and tooth loss owing to periodontitis. Grading covers three levels (grade A – low risk, grade B – moderate risk, grade C – high risk for progression), in addition to variables relating to periodontitis advancement, general health state, and other exposures such as smoking or level of metabolic control in diabetes. As a result, grading allows the physician to add unique patient characteristics into the diagnosis, which is critical for thorough case management [3].

Exfoliated (mucosa) and non-shedding solid surfaces (tooth surfaces), as well as fluid saliva, make up the oral cavity. As a result, oral bacteria colonizing a variety of surfaces have a high degree of spatial specificity[5].The importance of microorganisms in dental plaque, as well as plaque's crucial role in the etiopathogenesis of periodontal disease, is well understood.As a result, oral infection control is crucial in clinical practise [6].

Periodontal pathogens, particularly gram-negative anaerobic bacteria like Aggregatibacter actinomycetemcomitans and red complex bacteria like Porphyromonas gingivalis, Tannerella forsythia, and Treponemadenticola, are obviously responsible for PD.These gram-negative anaerobic bacteria have been shown to contain virulence factors, such as producing inflammatory mediators in the host, causing collagen breakdown in connective tissue due to particular enzymes, and alveolar bone resorption [7].

According to 16s rRNA sequencing of the oral microbiome, in periodontitis patients' disease areas, the abundance of periodontitis-associated species such as P. gingivalis, Treponemadenticola, Tannerella forsythia, Actinobacillusactinomycetemcomitans, and Fusobacterium nucleatum is significantly high [8].

The prevalence of periodontal infections is estimated to vary greatly according to geographic region and population.Differences in the prevalence and distribution of periodontal microbiome may play animportant role in putting some people at a higher risk of infection and PD than others, so understanding the epidemiology of PD is critical for PD prevention and therapy [9].Several brand new researches have been accomplished to determine the prevalence of certain periodontal pathogens in adults with periodontitis from various populations and nationalities, but none have been conducted on Egyptian populations, and only a few studies have been published that compare multivariate periopathogen levels in diverse periodontal diseases.So, the aim of the current study was to detect the prevalence of periodontal microbiota in Egyptianpopulation with periodontal diseases.

SUBJECTS AND METHODS

Study design

The study was on 100 patients selected consecutively from those patients referred to the Department of Oral Medicine, Periodontology, Diagnosis and Radiology, Faculty of Dental Medicine for Girls, Al- Azhar University; seeking for periodontal treatment. Patients were selected according to the criteria of new Periodontal Diseases Classification [10]. Other systemic diseases that impact the periodontium or interfere with periodontal treatment were not present in the patients.No periodontal therapy, systemic antibiotics or non-steroidal anti-inflammatory agents during the preceding 3 months and no Pregnant women were included in the study. All subjects were told about the nature and benefits of their involvement in the study prior to any procedure.All of the patients signed a consent form that was satisfactory to them.Finally, the Ethical Committee Faculty of Dental Medicine for Girls, Al-Azhar University approved the study and gave it the code (REC-ME-21-05).

Sample Size:

Sample size calculation has been done according to the following equation [11] : 4P*Q/d2

Where P is the prevalence, Q = 100- P, and D= allowable error (15% of P).

Study participants:

Patients were subdivided into 5 groups: Group I: 20 patients with healthy periodontium. Group II: 20 patients with periodontal disease diagnosed as stage I. Group III: 20 patients with periodontitisdiagnosed as stage II. Group IV: 20 patients with periodontal disease diagnosed as stage III. Group V: 20 patients with periodontal disease diagnosed as stage IV.Scaling and root planning, motivation, and oral hygiene instructions were first given to each patient as part of the cause-related therapy.Clinical parameters including Probing pocket depth, Clinical attachment level, Plaque Index (PI) and gingival index (GI) were measured (Fig. 1).

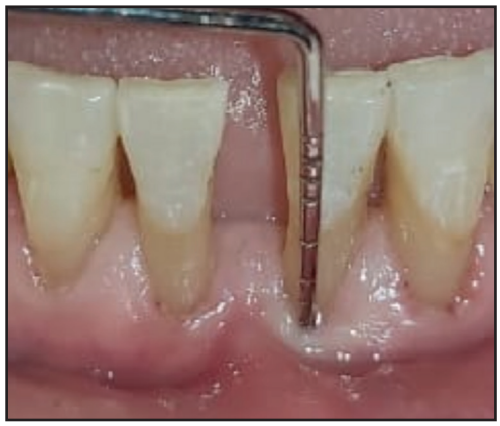

Figure (1) Clinical photo showing PD (1 mm) and CAL (5mm).

Collection of Subgingival Biofilm:

The teeth with the deepest pocket were first identified, isolated with cotton roll, and cleansed of supragingival plaque, as required by the study. A sterile curette was used to collect a pooled subgingival plaque sample from the deepest periodontal pockets. The samples were stored frozen at -800 degrees Celsius until they were analyzed [12].

Bacterial identification using PCR for the 5 bacteria

Five periodontal pathogens Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis(Pg), Treponemadenticola (Td), Tannerellaforsythia (Tf), and Fusobacterium nucleatum(Fn) were studied using microbial analysis. A commercially available real-time PCR kit was used.TaqMan probes were used in real-time PCR, and primers were created to amplify specific target genes from each bacterium. The probes' specificity was tested on all of the microorganisms in this investigation.

RESULTS

The results were statistically analysed using a one-way ANOVA followed by a post hoc test. The Pearson Correlation coefficient test was used to investigate the relationship between clinical and molecular data. Pearson correlation is a statistical test that determines the strength and direction of a linear relationship between two variables. Its value always ranges between -1 (strong negative relationship) and +1 (strong positive relationship), and the test criteria can be classified using the Likert scale.

Clinical assessment, Table 1:

For CAL and Max PD, the means were increased by increasing the stage number.For CAL and Max PD, there was a statistically significant difference between the healthy group and all stages.For CAL and Max PD, there was no statistically significant difference between Stage III and Stage IV.For CAL and Max PD, The overall P-value for intragroup comparison was highly significant.For Plaque Index, the majority of patients recorded a score 2, and the highest value was recorded in stage IV (60%). For Gingival Index, the majority of patients recorded a score 1, and the highest value was recorded in the healthy group (70%).

Molecular assessment, Table 2:

For all kinds of bacteria the mean of real timePCR increased by increasing the stage number. There was statisticallysignificant difference between the healthy group and all stages. There was no statistically significant difference between Stage I and Stage II. The overall P-value for intragroup comparison was highly significant except A.A was not significant. From the overall mean of real timePCRfor every kind of bacteria, we can arrange the sequence of bacteria as the following: T.f, P.Gingivalis, T.d, F.n, A.A

Table (1) *Comparative evaluation of clinical variables in different stages.*

Group		Healthy	Stage I	Stage II	Stage III	Stage IV	P value
Maximum probing depth		1.85±0.75[a]	3.80±0.62[b]	4.55±0.60[bc]	5.30±1.26[cd]	6.05±1.70[d]	0.00
Clinical attachment loss		0.00±0.00[a]	1.65±0.49[b]	3.65±0.49[c]	6.05±1.28[d]	6.90±1.86[d]	0.00
Plaque Index	Score 1	60%	60%	50%	30%	25%	
	Score 2	40%	40%	45%	50%	60%	
	Score 3	0%	0%	5%	20%	15%	
Gingival Index	Score 0	25%	0%	0%	0%	0%	
	Score 1	70%	50%	40%	20%	25%	
	Score 2	5%	40%	45%	70%	40%	
	Score 3	0%	10%	15%	10%	35%	

Small letters for intragroup comparison (healthy vs. stage I vs. Stage II …..etc.) and means with different superscript are statistically significant different at P ≤ 0.05

Table (2) *Comparative evaluation of molecular variables in different stages.*

Group	Healthy	Stage I	Stage II	Stage III	Stage IV	P value
P.Gi	117.6±42.20[a]	258.2±90.05[b]	280.7±97.71[b]	328.95±85.05[bc]	382.75±96.02[c]	0.00
A.A	2.05±0.22[a]	3.98±0.75[b]	4.35±0.52[b]	4.55±0.34[b]	4.60±0.36[b]	0.853
T.d	7.35±3.89[a]	29.90±13.18[b]	35.30±15.78[b]	35.35±15.96[b]	36.90±16.28[b]	0.00
T.f	134.30±32.56[a]	377.60±40.01[b]	390.55±34.36[b]	423.85±27.03[c]	439.50±30.97[c]	0.00
F.n	8.65±3.09[a]	16.96±4.31[b]	17.88±4.46[b]	18.19±6.53[b]	21.27±5.67[b]	0.00

– Small letters for intragroup comparison (healthy vs. stage I vs. Stage II …..etc.) and means with different superscript are statistically significant different at P ≤ 0.05

Correlation between Clinical data and Molecular data

1. Correlation between P. Gingivalis and Clinical variables, table 3:

There was a weak positive correlation between P.Gingivalisreal time PCR mean and Plaque Index (PI) (r = 0.240), and this correlation was statistically significant (P > 0.05). There was a moderate positive correlation between P.Gingivalisreal time PCR mean and Gingival Index (GI) (r = 0.408), and this correlation was statistically highly significant at the 0.01 level (P < 0.01& confidence 99 %). There was a moderate positive correlation between P.Gingivalisreal time PCR mean and Clinical Attachment Loss (CAL) (r = 0.555), and this correlation was statistically highly significant at the 0.01 level (P < 0.01& confidence 99%). There was a moderate positive correlation between P.Gingivalisreal time PCR mean and Maximum Probing Depth (r = 0.646), and this correlation was statistically highly significant at the 0.01 level (P < 0.01& confidence 99 %).

Therefore, there was a clear positive (weak to moderate) correlation between P.Gingivalisreal time PCR mean and Clinical variables.

Table (3) *Correlation between Perpharmonas Gingivalis P. Gingival isreal time PCR mean and clinical variables.*

Clinical variables	r**	P-Value	Correlation type
Plaque Index (PI)	0.240	0.0160	**Weak positive**
Gingival Index (GI)	0.408	0.000[HS]	**Moderate positive**
Clinical attachment loss (CAL)	0.555	0.000[HS]	**Moderate positive**
Maximum probing depth (Max PD)	0.646	0.000[HS]	**Moderate positive**

*- ** Pearson Correlation value -[HS]Highly significant (Correlation is significant at the 0.01 level).*

Correlation between Tannerella forsythia (T.f)real time PCR meanand Clinical variables, table 4:

There was a weak positive correlation between T.freal time PCR mean and Plaque Index (PI) (r= 0.228), and this correlation was statistically significant (P > 0.05) . There was a moderate positive correlation between T.freal time PCR mean and Gingival Index (GI) (r = 0.516), and this correlation was statistically highly significant at the 0.01 level (P < 0.01& confidence 99 %). There was a moderate positive correlation between T.freal time

PCR mean and Clinical Attachment Loss (CAL) (r = 0.738), and this correlation was statistically highly significant at the 0.01 level (P < 0.01& confidence 99 %). There was a moderate positive correlation between T.freal time PCR mean and Maximum Probing Depth (r = 0.724), and this correlation was statistically highly significant at the 0.01 level (P < 0.01& confidence 99 %). Therefore, we can briefly say that there is a clear positive (weak to moderate) correlation between Tannerella forsythia (T.f) real time PCR meanand Clinical variables.

Table (4) *Correlation between Tannerella forsythia (T.f) real time PCR meanandclinical variables.*

Clinical variables	r**	P-Value	Correlation type
Plaque Index (PI)	0.228	0.022	**Weak positive**
Gingival Index (GI)	0.516	0.000^{HS}	**Moderate positive**
Clinical attachment loss (CAL)	0.738	0.000^{HS}	**Moderate positive**
Maximum probing depth (Max PD)	0.724	0.000^{HS}	**Moderate positive**

*** Pearson Correlation value*

-^{HS}Highly significant (Correlation is significant at the 0.01 level).

DISCUSSION

Some species are widely known for their periodontal pathogenicity as a result of decades of oral microbiology research. New suspected periodontal infections have been discovered elevated in periodontitis patients using culture-independent and molecular-based techniques to bacterial identification. However, few studies have looked into the link between the severity of periodontal disease and the presence of these microorganisms. The initial stage in the quest for biomarkers that can predict the start of the disease is to identify bacteria that are strongly associated with periodontal condition [13].

The disease's prevalence and severity appear to differ significantly between industrialized and developing countries, as well as geographical areas. This variance could be due to the disease's etiology, as well as demographic and cultural factors[14].As a result, gathering knowledge on the anaerobes linked to PD in various locales is critical. The aim of this studywas detection and prevalence of periodontal microbiota in a group of Egyptians.

Periodontitis has a complicated and variable etiology. Several of the over 700 bacterial species that reside in the subgingival plaque have been linked to periodontitis. The most prevalent periodontopathogens are P. gingivalis, T. denticola, and T. forsythia, as well as F. nucleatum and A.A.[12].

Clinical periodontal parameters used in the present study assessing the periodontal conditions are those commonly used in clinical trials; including the Plaque Index (PI), Gingival Index (GI), Pocket Depth (PD),and Clinical Attachment Level (CAL). These indices have proved to be useful means of screening the periodontal condition. This traditional method of diagnosis is still recommended today, in which tooth by tooth charting is used to identify various clinical factors to locate loss of attachment and any tooth mobility, as well as measure the depth of periodontal pockets, gingival recessions, and the height of keratinized gingival tissue[15].

The majority of oral infections are non-cultivable, which is why we used molecular identification methods like PCR in this study. Several molecular techniques, including PCR-based methods, have been investigated to detect potential periodontal microbiomes [16].

The current findings help to determine the epidemiological distribution of five major periodontopathogens (Pg, Tf, Td, Fn, and Aa) discovered by PCR in adult Egyptian periodontal disease patients. The real-time PCR results show that The less prevalent microorganism was *AA* that found more rarely in Egyptian population, while P. gingivalis and T. forsythia were the most frequent and in higher amount. The PD pathogens are related with the progression and severity ofthe disease.

A higher occurrence of T. forsythia, was demonstrated in the periodontitis patients of Egyptian population. This finding contrasts with the findings of other studies[16]who reported lower detection levels in sites of periodontitis than this study.while the current results are in agree with other studies [14].

A higher occurrence of P. gingivalis, was demonstrated in the periodontitis patients of Egyptian population.This finding contrasts with the findings of previous studies [14,17] who reported lower detection levels in sites of periodontitis than this study,while the current results are in agree with previous study [16].

In our study, A.a was detected in alow proportion in the periodontitis patients of the Egyptian population. This finding contrasts with the findings of other researchers [14] while our results are in agree with other researches [16].

The differences that have been reported on the subgingival microbial composition in periodontitis patients among the Egyptian population and other different geographical and ethnic populations may be due to the variety of techniques that have been utilized, including microbiological cultivation methods and different molecular techniques as well as the differences in environment, study designs, sample sizes, sample selection and subgingival plaque sampling methods. Factors such as genetic background, diet, culture, healthcare practices, socioeconomic status, oral hygiene procedures and access to dental care have been attributed to be likely causes for the differences in the subgingival plaque microbial composition among different subjects.

CONCLUSION

This study shows the distribution of subgingival periodontal pathogens in chronic periodontitis in Egyptian population.

RECOMMENDATIONS

Further studies among the Egyptian population in a larger sample size are required to assess differences in the pattern of distribution of these and other organisms and their strains, and their role in the pathogenesis of periodontitis.

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DECLARATION STATEMENT

There are no conflicts of interest declared by the authors.

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