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PREVALENCE OF PERIODONTAL PATHOGENS AMONG ITALIAN PATIENTS WITH CHRONIC PERIODONTITIS: A RETROSPECTIVE STUDY ON 2992 PATIENTS

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SUMMARY
Purpose. The aim of the present study was to evaluate the prevalence of some periodontal pathogens in Italian adults with chronic periodontitis.
Materials and methods. The sample consisted of 2992 patients with a clinical diagnosis of chronic periodontitis, based on the criteria of the American Academy of Periodontology, sampled in the period 2013-2016: 2108 patients were from Northern, 690 from Central and 194 from Southern Italy. *Porphyromonas gengivalis, Treponema denticola,* and *Tannerella forsythia* were investigated in all patients of the present study, while *Campylobacter rectus, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* only in 2514 (84%) patients.
Subgingival plaque samples of the four sites of greatest probing depth in each patient were used to obtain subgingival microbiota and then processed by quantitative polymerase chain reaction.
Results. Periodontal pathogens had the following presence respect to all amount of patients: *Aggregatibacter actinomycetemcomitans* 16.1%, *Campylobacter rectus* 73.4%, *Fusobacterium nucleatum* 93.8%, *Porphyromonas gengivalis* 65.5%, *Treponema denticola* 66.4%, and Tannerella forsythia 72.7%. There are no significant statistical differences among geographic areas both for the total bacterial and the single species except for *T. Denticola* and *C. Rectus,* which prevalence was significantly higher in Southern Italy (P value <.05). The other investigated species were equally distributed among different regions. *A. actinomycetemcomitans* was the rarer species detected in this study, while *F. nucleatum* was the commonest. No differences among areas where observed as regard of the mean bacterial load except for F. Nucleatum whose prevalence in Northern Italy was lower then both in Central and Southern Italy (P value <.05).
Conclusions. The results of our study didn't show different geographic distribution of periodontal pathogens among Italian population of the three areas investigated. The homogeneity of the results could be related to genetic and environmental factors.

Key words: periodontitis, pathogens, bacteria, population, oral disease.

Introduction

Periodontal disease (PD) is one of the most prevalent chronic diseases in the world and in the Italian population. Periodontal disease (PD) is characterized as a chronic inflammatory condition affecting the periodontal tissues and dental plaque seems to play an essential role in the pathogenesis of this condition (1, 2).
PD is clearly caused by periodontal pathogens, in particularly some gram-negative anaerobic bacteria, such as *Aggregatibacter actinomycetemcomitans* (AA) and red complex bacteria (*Porphyromonas gingivalis*, PG –, *Tannerella forsythia*, TF and *Treponema denticola*, TD)

(3, 4). These gram-negative anaerobic bacteria have been demonstrated potential virulence factors, inducing host inflammatory mediators, leading to destruction of collagen of connective tissue due to specific enzymes, and alveolar bone resorption (5, 6).
The prevalence of periodontal pathogens is estimated very different in relation to geographic location and different populations (7, 8). Differences in prevalence and distribution of periodontal pathogens may play a major role in placing some population at a greater risk of infection and PD than others, so it is very important to know the epidemiology of PD for prevention and treatment of this disease (9, 10).
Presence and distribution of periodontal pathogens were investigated in Italian population (11-14). In these investigations, *Fusobacterium nucleatum* (FN) resulted the most frequently detected (95%) while TF showed the highest load. AA was the less represented bacterium for load and presence.
The aim of this investigation is to perform an epidemiological study about the presence and distribution of the most common bacteria in Italian adults with chronic PD.

Materials and methods

Patients

This epidemiological study was performed on patients of different private practices of North, Central and South of Italy between January 2013 and December 2016. The three areas included Italian regions according to italian institute of statistics (ISTAT) (www.istat.it/it/archivio/regioni). The sample comprised 2992 patients: 2108 from North (70.0%), 690 from Central (23%) and 194 of South (7%) of Italy. *Porphyromonas gengivalis*, *Treponema denticola*, and *Tannerella forsythia* were investigated in all patients of the present study, while *Campylobacter rectus*, *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* only in 2514 (84%) of patients. All patients were diagnosed of PD according to AAP (American Academy of Periodontology) criteria, and selected on the basis of the following inclusion criteria: age >18 yrs., probing depth of 3 mm or more. These criteria state that the patient must have at least one site with a probing depth and clinical attachment loss ≥ 4 mm. Subgingival plaque samples of the four sites of greatest probing depth in each patient were used to obtain subgingival samples.

Microbiological evaluation

Samples for microbiological analysis were collected from the four sites of greatest probing depth in each quadrant, with sterilized n. 60 paper tips inserted to the depth of the pocket, left in place for 20 seconds, transferred to a sterile tube and sent for subsequent DNA extraction and polymerase chain reaction (PCR) analysis. Table 1 reported probe and primer sequences used for the amplification.

DNA extraction

After collection, paper probes were processed for bacterial DNA extraction, by using the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St., St. Louis, MO, USA) and following the manufacturing procedures. Briefly, to isolate DNA, samples were incubated with lysozyme in a specific lysis buffer and, subsequently with proteinase K. Later, extracted DNA was purified by spin-column method.

Real-Time Polymerase Chain Reaction

Real-Time Polymerase Chain Reaction using the absolute quantification with the standard curve method performed bacterial quantification.

Table 1 - Probe and primer sequences use for periodontal bacteria amplification.

Periodontal bacteria	Primer sequence 5' -> 3'	Probe sequence
Aa	f-ACCTTACCTACTCTTGACATCCGAA r-ATGCAGCACCTGTCTCAAAGC	AAGAACTCAGAGATGGGTTTGTGCCTAGG
Pg	f-CGCGTGAAGGAAGACAGTCC r-CGATGCTTATTCTTACGGTACATTCA	TACGGGAATAACGGGCGATACGAGTATTG
Tf	f-CAGCGATGGTAGCAATACCTGTC r- TTCGCCGGGTTATCCCTC	TGAGTAACGCGTATGTAACCTGCCCGC
Td	f-AGCTACGGCTCCGCTTCAG r-GATACCCATCGTTGCCTTGGT	AGCTAATGGGACGCGGGCCCAT
Fn	f-AGGGTGATCGGCCACAAG r-CACAGAATTGCTGGATCAGACTCT	ACACGGCCCTTACTCCTACGGGAGG
Cr	f-TGACGCTAATGCGTGAAAGC r-CTCGACTAGCGAAGCAACAACTAG	TACCCTGGTAGTCCACGCCCTAAACGA
TBL	f- TGGAGCATGTGGTTTAATTCGA r-TGCGGGACTTAACCCAACA	CACGAGCTGACGACARCCATGCA

Aa = *Aggregatibacter actinomycetemcomitans*; Pg = *Porphyromonas gingivalis*; Tf = *Tannerella forsythia*; Td = *Treponema denticola*; Fn = *Fusobacterium nucleatum*; Cr = *Campylobacter rectus*; TBL = Total bacterial load.

Primers and probes oligonucleotides for *P. gingivalis* and *T. forsythia* were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1).
For the quantitative analysis, plasmids (Eurofin MWG Operon, Ebersberg, Germany) containing the specific DNA target sequence were employed as standard.
All reactions were performed in duplex, in 20ul final volumes; with 2X TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA) and 50nM concentration of each primers and 200nM of the probes. Amplifications were carried out by using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Chi-square test was performed to compare the prevalence of each bacterial species in the patient groups. SPSS program was used to perform statistical tests. A 5% level of significance and 95% confidence interval were used for all tests.

Results

The observed load of each investigated bacterial species in the periodontal pockets of patients was reported in Table 2. *Porphyromonas gengivalis*, *Treponema denticola*, and *Tannerella forsythia* were investigated in all patients of the present study, while *Campylobacter rectus*, *Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans* only in 2514 (84%) patients. Periodontal pathogens have the following presence respect to all amount of patients: *Aggregatibacter actinomycetemcomitans* 16.1%, *Campylobacter rectus* 73.4%, *Fusobacterium nucleatum* 93.8%, *Porphyromonas gengivalis* 65.5%, *Treponema denticola* 66.4%, and *Tannerella forsythia* 72.7%. There are no significant differences in presence among the different geographic areas both for presence and distribution except for *T. Denticola and C. Rectus w*hich prevalence was significantly higher in Southern Italy. The other investigated species were equally distributed among different regions.
A. actinomycetemcomitans was the rarer species

Table 2 - Mean amounts of specific bacterial species detected in periodontal pockets of periodontitis patients.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
AA	2514	,00	606731,00	2754,3500	21188,76
CBT	2992	65,00	4,2E+08	2897597	1,2E+07
CR	2514	,00	3909041	48174,84	181437,8
FN	2514	,00	9771033	243519,1	627376,0
PG	2992	,00	8976221	137964,1	506212,6
TD	2992	,00	2744233	74998,36	220887,8
TF	2992	,00	7735814	37262,02	206079,6
Valid N (listwise)	2514				

detected in this study, while *F. nucleatum* was the commonest. No differences among areas where observed as regard of the mean bacterial load except for *F. Nucleatum* whose prevalence in Northern Italy was lower than both Central and Southern Italy (Table 3, P value < 0.05).

Discussion

The present epidemiological study established the presence and distribution of PD bacteria in a large sample of Italian population, and investi-

Table 3 - Relative amounts of specific bacterial species detected in periodontal pockets of periodontitis patients.

Report

AREA		AA	CBT	CR	FN	PG	TD	TF
CENTRO	Mean	1984,4339	3681511	41738,89	282819,3	134343,6	83188,39	39160,13
	N	567	690	567	567	690	690	690
	Std. Deviation	17563,81	2,1E+07	153341,3	818138,4	511871,0	240731,5	193038,0
NORD	Mean	2717,1001	2621102	47578,83	223502,3	136695,3	72171,46	36620,55
	N	1759	2108	1759	1759	2108	2108	2108
	Std. Deviation	22392,82	7611808	185808,9	563732,3	478543,1	218703,5	217046,6
SUD	Mean	5424,9096	3113832	73161,82	312275,5	164627,7	76585,85	37481,19
	N	188	194	188	188	194	194	194
	Std. Deviation	19407,50	4651181	214227,9	512489,4	731455,8	163549,3	103988,5
Total	Mean	2754,3500	2897597	48174,84	243519,1	137964,1	74998,36	37262,02
	N	2514	2992	2514	2514	2992	2992	2992
	Std. Deviation	21188,76	1,2E+07	181437,8	627376,0	506212,6	220887,8	206079,6

Aa = *Aggregatibacter actinomycetemcomitans*; Pg = *Porphyromonas gingivalis*; Tf = *Tannerella forsythia*; Td = *Treponema denticola*; Fn = *Fusobacterium nucleatum*; Cr = *Campylobacter rectus*.

gates the differences among geographical areas. *AA* was found more rarely in Italian population, while *F. nucleatum* was the most frequent and in higher amount. The PD pathogens are related with the progression and severity of the disease. *F. nucleatum* is estimated one of the most abundant gram-negative anaerobes in patients with PD. The presence of *F. Nucleatum* is the main cause of PD worsening. The deeper is the pocket, more abundant is the amount of *F. Nucleatum*.

Differences in prevalence of TD and CR were observed. In addition difference in relative amounts of FN was detected among the three Italian areas. *F. Campilobacter Rectus* and *Treponema denticola* presence was significantly higher in Southern Italy. These results could be related to different genetic and environmental factors in the three areas. The study of presence and distribution of periodontal pathogens can be useful to tailor the best therapeutic protocol in each patient leaving in different geographic area (6, 15-21). Infection can happen with high frequencies in bone regeneration (22-25) also after cancer resection (26-30). In some pediatric conditions can be useful to have a low bacterial loading especially in syndromic conditions (31-33).

In addiction, further studies should be performed to establish the relationship between periodontal patogens and peri-implantitis. Tooth replacement with implants is a well-known technique used worldwide in the last 40 years (34-71). Bacteria of periodontal disease may worsen the implants survival rate (52, 53, 55, 72-86), or influence the surgical success in bone regeneration (22-25) or affecting young patients (87-92). Previous epidemiological studies in Italian population didn't report differences in PD pathogens presence and distribution. It may be explained by the homogeneity of periodontal diagnostic criteria. PD is one of the most diffuse chronic diseases in Italy, both in moderate to severe degree, and this fact explain the great amount of PD pathogens found in our study. In fact, the deeper is the pocket of the microbiological sample, the most aggressive are the bacteria, leading to tooth mobility and loss.

In conclusion, our study showed there are no significant differences in presence and distribution among the different geographic areas except for *T. Denticola and C. Rectus w*hich prevalence was significantly higher in Southern Italy. In addition, we found a similar bacterial load in patients living in different Italian geographic areas, with the only exception of *F. Nucleatum*.

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