Periodontal Pockets Microbiota qPCR Analysis at Different Stages of Periodontitis

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Background

Periodontitis is a common chronic inflammatory disease of tooth-supporting tissues caused by multibacterial infection [1, 2]. It has been shown that periodontitis patients carry higher number of disease-associated bacteria than healthy ones [3, 4]. But there is a few data on periopathogens profile and those fractions in periodontal pocket microbiocenosis.

Aims

The aim of this study was to evaluate the association between periodontal microbiota and chronic periodontitis (CP) by comparing periodontal pocket bacterial profile of patients with generalized chronic periodontitis (at different stages) and healthy controls.

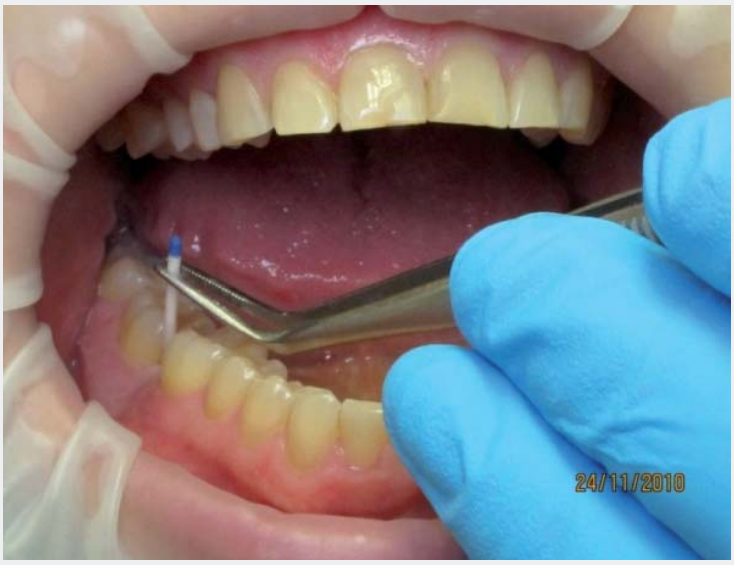

Fig 1. Periodontal pocket samples taking

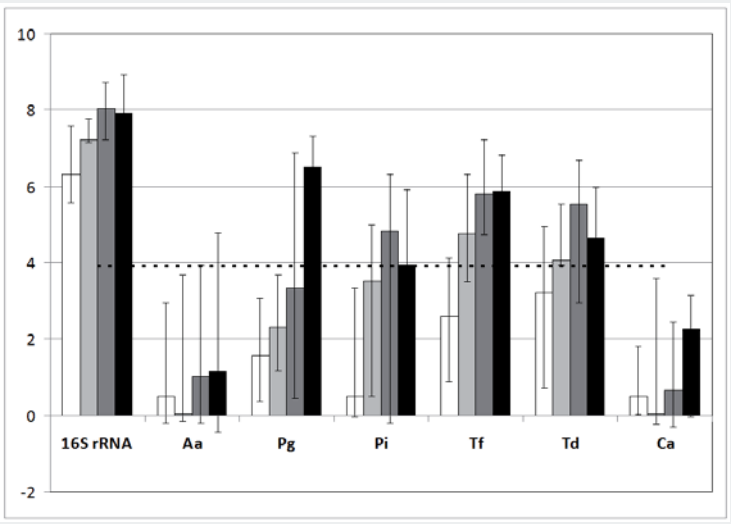

Fig 2. The gDNA normalized relative amount of all pathogens and total bacterial mass in control (white), light CP (light gray), medium CP (dark gray) and severe CP (black) columns (medians with 25-75 percentile). gDNA level is short dashes. Y-axis is a common logarithm of target DNA in the sample (conventional units). 16S rRNA – total bacteria amount, Aa – A.actinomycetemcomitans , Pg – P.gingivalis, Pi – P.intermedia, Tf – T.forsythensis, Td – T.denticola, Ca – C.albicans.

Methodology aspects

A total of 308 patients were examined. 208 patients (70 light CP, 68 medium CP and 70 severe CP) were included in the case group, while 100 healthy individuals served as a control group (see Table 1). Periodontal pocket samples were taken by endodontic pins #25, in two replications for each patient (see Fig 1). DNA was extracted with silica-based DNA Extraction Kit Probe-GS (DNA-Technology, JSC).
Original qPCR assay and DTprime Real-Time PCR Cycler (DNA-Technology, JSC) were used to quantify six major periodontal pathogens - *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Treponema denticola, and Candida albicans* as well as total bacterial mass with standardization to human gDNA.

Table 1. Control and case groups age structure (%)

Age	Healthy controls (n=100)	Light CP (n=70)	Medium CP (n=68)	Severe CP (n=70)
up to 35	48,0	60,0	14,7	-
35 - 44	43,0	40,0	28,0	28,6
45 - 54	9,0	-	35,3	40,0
over 55	-	-	22,0	31,4

Results

Table 2 show the percentage of positive samples in case and control groups. We found that total bacterial mass increased with disease progression up to two orders for severe generalized chronic periodontitis (see Fig 2). The percent of pathogen in total bacterial mass is also vary with disease progression (see Table 3). Based on qPCR analysis of six periodontal pathogens during CP progression we propose four types of pathogen representation dynamics in total bacterial mass: 1) a decrease of pathogen portion (T. denticola); 2) a constant (A. actinomycetemcomitans); 3) an increase at the early stage of disease (P. intermedia, T. forsythensis) and 4) an increase at the late stages of disease (P. gingivalis, C. albicans). We believe that any particular type of pathogen representation dynamics depends on a role of given microorganism in the disease progression.

Table 2. The positive samples in case and control groups (%)

Pathogen	Healthy controls (n=100)	Light CP (n=70)	Medium CP (n=68)	Severe CP (n=70)
A.actinomycetem comitans	10,0	32,9	48,5	42,8
P.gingivalis	47,0	70,0	66,2	85,7
P.intermedia	33,0	70,0	58,8	71,4
T.forsythensis	53,0	80,0	97,1	100
T.denticola	60,0	80,0	82,3	82,9
C.albicans	10,0	40,0	38,2	60,0

Table 3. The approximate amount of pathogen in total bacterial mass (%)

Pathogen	Healthy controls (n=100)	Light CP (n=70)	Medium CP (n=68)	Severe CP (n=70)
P.gingivalis	0,003	0,002	0,003	4
P. intermedia	0,0003	0,03	0,06	0,01
T. forsythensis	0,05	0,6	0,6	0,7
T. denticola	0,2	0,1	0,06	0,04
C. albicans	0,0003	0,00001	0,00005	0,002

References

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