Detection and Quantification of *Porphyromonas gingivalis* from Saliva of Schizophrenia Patients by Culture and Taqman Real-Time PCR: A Pilot Study

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Abstract: *P. gingivalis* is a periodontopathogen implicated in a number of systemic diseases, particularly cardiovascular disease. Little, if any, is known about the prevalence and quantity of this organism in the mouth of patients with schizophrenia who are, nevertheless, known to have poor oral health and die early from cardiovascular disease. Aim: to estimate the prevalence and quantity of *P. gingivalis* in saliva of schizophrenia patients compared to non-psychiatric controls and to correlate the quantity of *P. gingivalis* with the severity of psychopathology of schizophrenia. Materials and methods: Thirty five consecutive attendees of the out-patients clinic of a psychiatric Hospital in Jeddah, with a diagnosis of schizophrenia, were assessed by the Positive and Negative Syndrome Scale (PANSS) and compared with 35 non-psychiatric controls, in terms of the prevalence and quantity of *P. gingivalis* in their saliva. For this purpose, anaerobic culture and real-time PCR with TaqMan probe were used. Results: Real-time PCR results were matching those obtained with anaerobic culture in 95.7% of cases. Using Real-time PCR, *P. gingivalis* was detected in 25 patients (78%) and 6 controls (17%) (p=0.000). The *P. gingivalis* median (range) number of copies in salivary samples of patients and controls were $5.3x10^7$ (0- 2.73^{10}) and $1.91x10^5$ (0- 6.81^7), respectively (p=0.009). Also, the *P. gingivalis* levels were significantly positively correlated with the scores on all the PANSS scales. Conclusion: real-time PCR, in confirmation of the results of quantitative culture, demonstrated (a) significantly higher prevalence and quantity of *P. gingivalis* in saliva of schizophrenia patients compared to non-psychiatric controls and (b) positive correlation between quantity of *P. gingivalis* cells and severity of psychopathology of schizophrenia. Hopefully, the results of this pilot study will encourage further research into the relationships between oral microbiota and schizophrenia. Real-time PCR is a promising tool in this area. Hopefully too, some preventive dental programs will become an integral part of psychiatric management to meet the need of this vulnerable group of population.

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1. Introduction

Bacterial inhabitants of the oral cavity comprise one of the most complex microbial community systems associated with human body. It has been estimated that more than 700 different bacterial species inhabit the human oral cavity[(1)]. These organisms, which live mainly in the saliva, have the ability to colonize the surfaces of the buccal cavity and to develop there in the form of complex biofilms[(2)]. They interact with each other and with their host tissues [(3)]. These interactions, however, are still not well understood. While the majority of oral microflora are normal/commensal bacteria, some of them are opportunistic pathogens responsible for the development of oral microbial infectious diseases such as dental caries and periodontitis [(4)]. Numerous studies have shown that these pathogens do not only cause chronic localized conditions, but may also increase the risk of various systemic diseases [(5-7)].

One of the most pathogenic species of the entire oral flora, and perhaps the most extensively studied at the molecular level is Porphyromonas gingivalis. It belongs to the family *Porphyromonadaceae*, order *Bacteroidales* in the phylum *Bacteroidetes*, formerly known as the *Cytophaga-Flavobacteria-Bacteroides* group[(8)], a black-pigmented Gram-negative, obligate anaerobic and asaccharolytic cocco-bacillus[(9)]. *P. gingivalis* is frequently found as a prominent component of the flora of subgingival lesions of adult patients with periodontitis[(10)]. Furthermore, *P. gingivalis* is incriminated in certain systemic conditions, such as atherosclerotic heart disease [(11)]. Pathogenicity of this organism is attributed to a large number of putative virulence factors, such as cysteine proteinases (gingipains), hemagglutinins, lipopolysaccharide (LPS), and fimbriae by which *P. gingivalis* is enabled to invade host tissues [(12)]. *P. gingivalis*, however, is among the late colonizers of the oral cavity, a process

that is facilitated by other microbial species that provide attachment sites, as well as supply growth substrates, and reduce oxygen tension to levels optimal for growth of *P. gingivalis*[8]. Colonization of *P. gingivalis* is influenced by saliva, which serves as a vector for its transmission and initial entry into oral environment[13]. Anchoring points for *P. gingivalis* fimbriae are also provided by salivary pellicle-coated tooth surfaces. *P. gingivalis* cells, then, enmesh into networks of intercellular communication with other oral prokaryotic cells and with eukaryotic cells[14].

In periodontitis patients, *P. gingivalis* can be detected in saliva, on the dorsum of the tongue, tonsils, buccal mucosa and gingiva, and other mucous membranes [15], whereas in periodontally healthy individuals, this organism is usually absent, or if present, it is in low numbers[16]. Studies suggest that periodontal disease can be minimized through maintenance of oral cleanliness[17]. However, non-compliance is a major issue [18]. Though it is a universal phenomenon, non-compliance appears to affect people with severe mental illness, such as schizophrenia, considerably more than other people [19]. In these patients, non-compliance is found to be as high with non-psychiatric drugs as with psychiatric medications[20], and is probably related to the severity of psychopathology[21, 22]. These patients are further disadvantaged not only by having higher rates of physical illnesses than those without schizophrenia, but also by experiencing greater difficulty in getting adequate health care[23]. Oral health, which is an integral part of the general health, and contributes to self esteem and quality of life, may have a low priority among these patients who are, nevertheless, liable to get dental problems[24]. General self-neglect associated with mental illness, misconceptions, fear of treatment, worry about the cost of treatment, inability to access dental services and the adverse-effects of medications are also among the most commonly cited barriers to dental care[25, 26].

To date, however, there has been relatively little research assessing orodental status of patients with schizophrenia. Most of these studies have focused on institutionalized chronic patients[27, 28] although the majority of patients are now living outside hospital. Oral health research about those attending outpatient psychiatric services is "almost non-existent"[29]. Moreover, approaches have been mostly restricted to a clinical descriptive level using self-report questionnaires[30, 24, 29] and/ or clinical dental examinations[31-34]. It may be rather surprising that, despite the availability and the researchers' extensive use of various procedures to examine oral microorganisms in various populations, no previous attempts have been made, as far as we know, to detect or quantify oral opportunistic pathogens, such as *P. gingivalis*, in patients with schizophrenia. These patients are particularly prone to cardiovascular disease [35] which is the chief cause of their excess premature mortality [36]. Ironically, oral infection with *P. gingivalis* has been also strongly associated with cardiovascular disease, even after adjustment for established cardiovascular risk factors [11]. However, it is not known whether there is any relation between *P. gingivalis* and schizophrenia, although researchers have tried, for more than a century, and still trying to find a role for infectious agents in triggering schizophrenia [37].

This cross-sectional pilot study aimed to estimate the prevalence and quantity of *P. gingivalis* in saliva of schizophrenia patients compared to non-psychiatric controls and to correlate the quantity of *P. gingivalis* with the severity of psychopathology of schizophrenia. We hypothesized that the severity of oral infection in patients with schizophrenia is related to the severity of psychopathology.

2. Material and Methods:

A total of 35 patients were recruited from consecutive attendees of the out-patients clinic of a large private psychiatric Hospital in Jeddah between January and July 2010, with a diagnosis of schizophrenia (F.20 of the ICD10)[38], aged 20 to 50 years, and for whom a stable regimen of antipsychotic medication was consistently prescribed for at least 3 months prior to recruitment.

The control group consisted of 35 subjects, individually matched for age and sex and randomly selected from companions of patients and from hospital employees and their acquaintances.

None of the subjects had current febrile acute infection, acute exacerbation of a chronic infection or an inflammatory disease, underlying hematologic, malignant, severe cardiac, liver or renal disease. None within the previous 12 weeks used antibiotics or had any dental or general surgery. Participants who had missing teeth and females who were pregnant or lactating were excluded from the study. Body Mass Index greater than or equal to 35 or less than or equal to 18 and blood pressure >160/100, were also exclusion criteria. Controls had no evidence of current or past history of any psychiatric disorder. A written informed consent was obtained from each participant.

Patients underwent a standardized psychiatric interview during which the ICD-10 diagnosis of schizophrenia was confirmed and the Positive and Negative Syndrome Scale (PANSS)[39] was applied by a trained psychiatrist. PANSS is a 30-item test, subdivided into three subscales: a Positive Scale (P) composed of seven items, a Negative Scale (N) composed of seven items and a General Psychopathology Scale (G) composed of 16 items.

Each item is rated on a seven point severity scale, from 1 (no evidence) to 7 (extreme).

Saliva sampling procedure

Saliva specimens were collected by expectoration into sterile calibrated medical cups. Saliva was put into Eppendorf tube, which was immediately frozen at –80 and stored until used in real-time PCR. For the detection of *P. gingivalis* by bacterial culture saliva samples were pooled in 1.5 ml of reduced transport fluid and were processed for cultivation under anaerobic conditions within 4 h of sampling. Samples were vortexed for 2 min and split. A total of 100 µl of the sample was used for culture by tenfold serial dilution in sterile phosphate-buffered saline solution.

Culture

Serial 10-fold dilutions were prepared, and the last three dilutions were used for plating on blood agar plates (Oxoid, Basingstoke, United Kingdom) supplemented with horse blood (5%; vol/vol), hemin (5 mg/liter), and menadione (1 mg/liter) and incubated anaerobically in jars filled by the evacuation-replacement method with a mixture of gases (85% N_2, 10% H_2, 5% CO_2) at 37°C for 7 to 14 days. The isolates were identified as *P. gingivalis* on the basis of Gram staining, anaerobic growth, having the typical colony color and morphology, lacking colony autofluorescence, positive hemagglutination with 3% sheep erythrocytes as well as the production of a set of metabolic enzymes (as tested with the Rapid ID kit 32A) and having a positive indole reaction. The total number of CFU of *P. gingivalis* in positive samples was determined.

Real-time PCR

Isolation of DNA

To extract DNA from the bacteria present in saliva, frozen suspensions were thawed and 100µl samples were used for automated DNA extraction and purification with the MagNA Pure DNA Isolation Kit III (Bacteria, Fungi; Roche Molecular Diagnostics). The protocol included 1 h of pretreatment with proteinase K (20 mg/ml) at 56°C. After isolation, the DNA was eluted in 100 µl of elution buffer.

PCR primers and probes

The 16S rRNA sequences of the genus *Porphyromonas* were selected. The sequence of the forward primer, , was 5′-GCGCTCAACGTTCAGCC-3′ (base pairs 612 to 628); the sequence of the reverse primer, , was 5′-CACGAATTCCGCCTGC-3′ (base pairs 664 to 679); and the sequence of the TaqMan probe, was 5′-CACTGAACTCAAGCCCGGCAGTTTCAA-3′ (base pairs 634 to 660) The primers and probes were purchased from Applied Biosystems (Foster City,California, USA).

Quantitative PCR assay

PCR amplification was performed in a total reaction mixture volume of 25 µl. The reaction mixtures contained 12.5 µl of 2× TaqMan universal PCR master mixture (PCR buffer, deoxynucleoside triphosphates, AmpliTaq Gold, an internal reference signal [6-carboxy-X-rhodamine], uracil *N*-glycosylase, $MgCl_2$; Applied Biosystems), 300 nM each *P. gingivalis*-specific primer, 100 nM *P. gingivalis*-specific probe. and 5 µl of purified DNA from plaque samples. Five microliters of the DNA extracted from *P. gingivalis* W83 was used to prepare the standard curve and as a positive control; the negative control was 5 µl of sterile H_2O.

The samples were subjected to an initial amplification cycle of 50°C for 2 min and 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. The degradation of the probe by the DNA polymerase in each elongation step induces an increase in fluorescence that can be monitored during PCR amplification. The fluorescence signal is normalized by dividing the reporter dye emission (6-carboxyfluorescein) by the emission of the passive reference (6-carboxy-X-rhodamine). The higher the starting copy number of the nucleic acid target is, the sooner a significant increase in fluorescence is observed. Hence, this parameter can be used to compare different amplification reactions. The number of bacterial copies was calculated assuming that the genome mass is equal to 2.37 fg (femtogram=10-15g)[40].

Statistical analysis:

Continuous data were expressed as mean (± standard deviation) or median (range) and were compared by use of Student's t-test, after testing for normality with a Kolmogorov–Smirnov-test and normalization by log-transformation where appropriate. Categorical data were expressed as frequencies or proportions and were analyzed with the two-tailed chi-square test. Correlations between data were analyzed using Pearson's coefficient. Data were analyzed using SPSS version 11.0.1. Software (SPSS for Windows, 2001). Two-tailed p values <0.05 were considered statistically significant.

3. Results:

Background characteristics of participants:

Background characteristics of patients and controls were not significantly different (table 1).

Severity of psychopathology in the patient group:

Mean (±SD) of scores of patients on the standard scales of PANSS are given in table 2.

Prevalence of salivary *P. gingivalis*:

P. gingivalis was more prevalent in salivas from patients than controls. *P. gingivalis* was detected in 78% (25 of 35) of the patient group but was found only in 17% (6 of 35) of the controls (table 3). For either group, no relationship was found between detection of *P. gingivalis* and gender, age or nationality. However, significant relationships were observed between *P. gingivalis* detection and being less educated, in a lower occupational position, not married, currently smoker and not a Miswak user (table 3).

Numbers of *P. gingivalis* cells:

Table (4) shows the results of absolute quantification of *P. gingivalis* cells determined in individual PCR runs. There is a significant difference between the number of *P. gingivalis* cells in salivary samples of patients and controls.

Relationship of the salivary *P. gingivalis* count with the severity of psychopathology in the patient group:

The salivary levels of *P. gingivalis* were significantly positively correlated with the scores on all the PANSS scales (table 5).

Comparison between PCR and culture:

Results obtained with real-time PCR were matching those obtained with anaerobic culture in 95.7% of cases (28 positive; 39 negative). A two-by-two contingency table summarizes the results (table 6). *P. gingivalis* was cultured from 28 (40%) of the 70 saliva specimens. All these culture-positive samples were also positive by the real-time PCR assay (100% sensitivity). In addition, 3 samples were positive for *P. gingivalis* by the real-time PCR but negative by culture. These samples were thawed and recultured for 14 days. Two of these samples yielded *P. gingivalis* after this prolonged culture. Of the 42 culture-negative samples, 39 were negative by PCR assay (90.3% specificity). None (0%) of the PCR-negatives was found to be culture-positive (table 6).

Table (1): Background characteristics of participants

	Patients N= 35	Controls N= 35	Significance
Gender:			
Male: N	21	21	χ^2 =0.000 ; *df*=1; p=1.000
Female: N	14	14	
Age (years):			
Mean (±SD)	29.9 (±8.9)	30.2 (±8.8)	*t*=0.163; *df*= 68; p=0.871
Age group:			
<30 years: N	22	21	χ^2 =0.060 ; *df*=1; p=0.806
>30 years: N	13	14	
Nationality:			
Saudi: N	16	17	χ^2 =0.057 ; *df*=1; p=0.811
Non-Saudi: N	19	18	
Education level:			
Intermediate or below: N	27	22	χ^2 = 1.701; *df*=1; p=0.192
Above intermediate: N	8	13	
Occupation:			
Higher: N	7	13	χ^2 = 2.520; *df*=1; p=0.112
Lower: N	28	22	
Marital status:			
Married: N	15	19	χ^2 =0.915; *df*=1; p=0.339
Unmarried*: N	20	16	
Currently smoking:			
Yes: N	22	14	χ^2 =3.660; *df*=1; p=0.056
No : N	13	21	
"Miswak"** habitual user:			
Yes: N	15	17	χ^2 =0.230; *df*=1; p=0.631
No : N:	20	18	

* Unmarried= Never married, divorced, separated and widowed. ** "Miswak" = "Sewak"=tooth cleaning stick

Table (2): Scores of patients on the Positive and Negative Syndrome Scale (PANSS)

Scale	Score
Total	
Mean (±SD)	82.2 (±13.0)
Positive	
Mean (±SD)	22.2 (±3.4)
Negative	
Mean (±SD)	22.1 (±3.1)
General psychopathology	
Mean (±SD)	38.1 (±9.7)

Table (3): Prevalence of *P. gingivalis* by real-time PCR*

	Real-time PCR		χ^2 (*df*=1)	p
	Positive N	Negative N		
All subjects				
Patients	25	10	20.902	0.000
Controls	6	29		
Gender				
Patients				
Male	16	5	0.583	0.445
Female	9	5		
Controls				
Male	3	18	0.302	0.583
Female	3	11		
Total				
Male	19	23	0.039	0.844
Female	12	16		
Age				
Patients				
<30 years	16	6	0.049	0.825
>30 years	9	4		
Control				
<30 years	3	18	0.302	0.583
>30 years	3	11		
Total				
<30 years	19	24	0.000	0.983
>30 years	12	15		
Nationality:				
Patients				
Saudi:	9	7	3.327	0.068
Non-Saudi:	16	3		
Control				
Saudi:	2	15	0.673	0.412
Non-Saudi:	4	14		
Total				
Saudi:	11	22	3.035	0.081
Non-Saudi:	20	17		
Education level:				
Patients				

Intermediate or below:	23	4	10.954	0.001
Above intermediate:	2	6		
Control				
Intermediate or below:	6	16	4.279	0.039
Above intermediate:	0	13		
Total				
Intermediate or below:	29	30	14.693	0.000
Above intermediate:	2	19		
Occupation:				
Patients				
Higher	1	6	14.000	0.000
Lower	24	4		
Control				
Higher	0	13	4.279	0.039
Lower	6	16		
Total				
Higher	1	19	17.514	0.000
Lower	30	20		
Marital status:				
Patients				
Married:	7	8	7.887	0.005
Unmarried*:	18	2		
Control				
Married:	0	19	8.599	0.003
Unmarried*:	6	10		
Total				
Married:	7	27	15.047	0.000
Unmarried*:	24	12		
Current smoker:				
Patients				
Yes:	20	2	11.014	0.001
No:	5	8		
Control				
Yes:	5	9	5.666	0.017
No:	1	20		
Total				
Yes:	25	11	19.014	0.000
No :	6	28		
Miswak" habitual user**				
Patients				
Yes:	8	7	4.212	0.040
No:	17	3		
Control				
Yes:	0	17	6.839	0.009
No:	6	12		
Total				
Yes:	8	24	8.886	0.003
No:	23	15		

* Number of subjects with *P. gingivalis* /Number of subjects tested (%)
** "Miswak" = "Sewak"=tooth cleaning stick

Table (4): Number of copies (median and range values) of *P. gingivalis* in salivary samples of patients and controls assessed by real-time PCR absolute quantification.

	Patients	Controls	Significance*
Median	$5.3x10^7$	$1.91x10^5$	t=2.694; df= 68; p=0.009
(Range)	(0- 2.73^{10})	(0- 6.81^7)	

*After normalization using logarithmic transformation

Table (5): Correlation coefficients between number of copies of *P. gingivalis* in salivary samples of patients and scores on the Positive and Negative Syndrome Scale (PANSS)

PANSS scale	r	p
Total	0.488	0.003
Positive	0.451	0.007
Negative	0.518	0.001
General psychopathology	0.431	0.010

Table (6): Detection of *P. gingivalis* by real-time PCR and anaerobic culture

Anaerobic culture result		Real-time PCR result*		
		Positive	Negative	Total
		N	N	N
Positive	N	28	0	28
Negative	N	3	39	42
Total	N	31	39	70

*Sensitivity= 100.0%; specificity= 90.3; Positive predictive value=100.0%; Negative predictive value=92.9%

4. Discussion:

We believe this study is the first to report a higher prevalence of the oral pathogen, *P. gingivalis*, in salivas from patients with schizophrenia than matched non-psychiatric controls. We used saliva because, as an oral circulating fluid, saliva is heavily laden with bacteria (108 – 109 cfu/mL)[41]. Previously, all 16S rRNA sequences of the genus *Porphyromonas* based saliva studies had utilized qualitative PCR. For the detection and quantification of *P. gingivalis* in saliva samples in the current study, however, we compared the results of a quantitative anaerobic culture method with those of a real-time TaqMan PCR assay, which is, unlike conventional PCR assays, less susceptible to PCR inhibition[42] and is suggested to provide a sensitive, efficient, and reliable approach to quantitation[43]. In keeping with this suggestion, we found the sensitivity, specificity, and positive and negative predictive values of the real-time PCR to be 100, 90.3, 100 and 92.9% respectively. We conclude, therefore, that real-time PCR confirms the results of quantitative culture of *P. gingivalis* and offers promising advantages with respect to the rapidity and sensitivity of detection of *P. gingivalis* in saliva samples. Until recently, however, very little attention has been given to the quantification of *P. gingivalis* in saliva, whether of psychiatric or non-psychiatric populations.

Our results demonstrated that in both patients and controls *P. gingivalis* detection was correlated with being less educated and in a lower occupational position. These results are consistent with previous studies which have shown that periodontitis is more common among people with low than with high socio-economic status, regardless of the indicator used[44]. Poverty, which raises the risk of schizophrenia, especially deficit schizophrenia[45], reduces the chance of receiving adequate dental care, and hence, could partly explain the results. Our finding that the *P. gingivalis* detection was more frequent among unmarried than married people in both patients and controls is also consistent with other studies showing higher susceptibility to various infections among single, widowed, and separated than married individuals, independent of other demographic factors[46]. Also, in keeping with other studies, which have indicated that smoking significantly increases the risk for the development of extensive and severe oral infections[47], we found significant correlation between *P. gingivalis* detection and current smoking in both patients and controls. Interestingly, habitual use of "Miswak" (the chewing stick or the traditional toothbrush in common use in Saudi Arabia and many Islamic countries) was negatively associated in this study with *P. gingivalis* detection. This should lend support to the few previous studies which have suggested that regular use of miswak is associated with good oral health[48-50].

Correlation coefficients of the salivary levels of *P. gingivalis* with scores on PANSS were determined in this study. The results showed that *P.*

gingivalis levels were significantly associated with the severity of schizophrenia psychopathology as expressed by PANSS scores, with negative symptoms presenting the strongest correlation. The achieved results were not unexpected, considering that negative symptoms, which include symptoms such as lack of initiative (PANSS: N2), apathy, anergy, or avolition (PANSS: N4), etc., would likely lead to a reduced self-care and poor dental health, far worse than that of members of the general population[24]. However, a cause and effect relationship between severities of the negative or other symptoms of schizophrenia and quantities of the oral pathogen should not be claimed by the present pilot study at least because of the limitation of its cross-sectional design.

Among participants we excluded from the study were those with evidence of cardiovascular disease. But this variable should have been important and interesting to investigate when relating to both *P. gingivalis* and schizophrenia. In addition, we did not report some rather relevant data such as details of medication history, general and dental clinical and radiographic examination findings. We did not assess the cognitive functions, although the central role of cognitive dysfunction in schizophrenia has been increasingly appreciated[51] while there have been some suggestions that cognitive impairment may be associated with periodontal disease[52, 53]. Also, the endocrine and metabolic status of the participants, despite relevance to both schizophrenia and oral infections with a possible confounding role, was not evaluated. One more limitation is the relatively small sample size.

In summary, we conclude within the limits of this study that the real-time PCR has confirmed the results of quantitative culture and demonstrated significantly higher prevalence and quantity of *P. gingivalis* in the saliva of patients with schizophrenia compared to non-psychiatric controls. Both real-time PCR and quantitative culture have also confirmed a positive correlation between quantity of *P. gingivalis* cells and severity of psychopathology of schizophrenia. This pilot study may be the first to report such findings. It is hoped, however, that the results will encourage further research into the relationships between oral microbiota and schizophrenia. Real-Time PCR, with its capacity to produce both qualitative and quantitative results, is a promising tool in this area. We should also hope that the need of the mentally ill for more dental care will be appreciated by all concerned and that some preventive dental programs will become an integral part of psychiatric management to meet the need of this vulnerable group of population.

Conflicts of interest: None

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5. References:

1. Aas, J.A., B. J. Paster, L.N. Stokes, I. Olsen and F.E. Dewhirst, 2005. Defining the normal bacterial flora of the oral cavity. Journal of Clinical Microbiology, 43(11):5721- 5732.
2. Socransky, S.S. and A.D. Haffajee, 2005. Periodontal microbial ecology. Periodontology 2000, 38:135-187.
3. Tian, Y., X. He, M. Torralba, S. Yooseph, K.E. Nelson, R. Lux, 2010. Using DGGE profiling to develop a novel culture medium suitable for oral microbial communities. Molecular oral microbiology, 25(5):357- 367.
4. Marsh, P.D. and R.S. Percival, 2006. The oral microflora--friend or foe? Can we decide? International Dental Journal, 56(4 Suppl 1):233-239.
5. Bascones-Martínez, A., M. Muñoz-Corcuera and J.H. Meurman, 2009. Odontogenic infections in the etiology of infective endocarditis. Cardiovascular & Hematological Disorders - Drug Targets, 9(4):231- 235.
6. Orrù, G., M.F. Marini, M.L. Ciusa, D. Isola, M. Cotti, M. Baldoni, 2006. Usefulness of real time PCR for the differentiation and quantification of 652 and JP2 Actinobacillus actinomycetemcomitans genotypes in dental plaque and saliva. BMC Infectious Diseases, 6:98.
7. Tomás Carmona, I., P. Diz Dios, J. Limeres Posse, A. González Quintela, C. Martínez Vázquez and A. Castro Iglesias, 2002. An update on infective endocarditis of dental origin. The Journal of Dentistry, 30(1):37-40.
8. Nelson, K.E., R.D. Fleischmann, R.T. DeBoy, I.T. Paulsen, D.E. Fouts, J.A. Eisen, *et al*., 2003. Complete genome sequence of the oral pathogenic Bacterium *porphyromonas gingivalis* strain W83. The Journal of Bacteriology, 185(18):5591- 5601.
9. Shah, H.N. and M. D. Collins, 1988. Proposal for Reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a New Genus, *Porphyromonas*. International Journal of Systematic and Evolutionary Microbiology, 38: 128-131.
10. Loos, B.G., D.W. Dyer, T.S. Whittam and R.K. Selander, 1993. Genetic structure of populations of *Porphyromonas gingivalis* associated with

periodontitis and other oral infections. Infection and Immunity, 61(1):204-212.
11. Seymour, G.J., P.J. Ford, M.P. Cullinan, S. Leishman and K. Yamazaki, 2007. Relationship between periodontal infections and systemic disease. Clinical Microbiology and Infection, 13 (Suppl 4):3-10.
12. Lamont, R.J. and H.F. Jenkinson, 1998. Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. Microbiology and Molecular Biology Reviews, 62(4):1244- 1263.
13. Hajishengallis, G., 2009. Porphyromonas gingivalis-host interactions: open war or intelligent guerilla tactics? Microbes and Infection, 11(6-7):637-45.
14. Lamont, R.J. and H.F. Jenkinson, 2000. Subgingival colonization by Porphyromonas gingivalis. Oral Microbiology and Immunology, 15(6):341-349.
15. Van Winkelhoff, A.J., U. Van der Velden, M. Clement and J. De Graaf, 1988. Intraoral distribution of black-pigmented Bacteroides species in periodontitis patients. Oral Microbiology and Immunology, 3: 83–85.
16. Griffen, A.L., M.R. Becker, S.R. Lyons, M.L. Moeschberger and E.J. Leys, 1998. Prevalence of Porphyromonas gingivalis and periodontal health status. Journal of Clinical Microbiology, 36: 3239–3242.
17. Ciancio, S., 2003. Improving oral health: current considerations. Journal of Clinical Periodontology, 30 (Suppl 5):4-6.
18. Miyamoto, T., T. Kumagai, J.A. Jones, T.E. Van Dyke and M.E. Nunn, 2006. Compliance as a prognostic indicator: retrospective study of 505 patients treated and maintained for 15 years. Journal of Periodontology, 77(2):223-232.
19. Surguladze, S., P. Timms and A. S. David, 2002. Teaching psychiatric trainees 'compliance therapy'. The Psychiatrist (Psychiatric Bulletin), 26:12-15.
20. Dolder, C., J. Lacro and D. Jeste, 2003. Adherence to antipsychotic and nonpsychiatric medications in middle-aged and older patients with psychotic disorders. Psychosomatic Medicine, 65:156-162.
21. Rettenbacher, M.A., A. Hofer, U. Eder, M. Hummer, G. Kemmler, E.M. Weiss and W.W. Fleischhacker, 2004. Compliance in schizophrenia: psychopathology, side effects, and patients' attitudes toward the illness and medication. The Journal of Clinical Psychiatry, 65(9):1211-1218.
22. Stanković, Z., D. Britvić, O. Vuković and T. Ille, 2008. Treatment compliance of outpatients with schizophrenia: patient's attitudes, demographic, clinical and therapeutic variables. Psychiatric Danub, 20(1):42-52.
23. Sartorius, N., 2007. Physical illness in people with mental disorders. World Psychiatry, 6(1): 3-4.
24. McCreadie, R.G., H. Stevens, J. Henderson, D. Hall, R. McCaul, R. Filik, *et al*., 2004. The dental health of people with schizophrenia. Acta Psychiatrica Scandinavica, 110(4):306-310.
25. Mirza, I., R. Day and M. Phelan, 2001. Oral health of psychiatric in-patients. The Psychiatrist (Psychiatric Bulletin), 25:143-145.
26. Nielsen, J., P. Munk-Jørgensen, S. Skadhede and C.U. Correll, 2010. Determinants of poor dental care in patients with schizophrenia: a historical, prospective database study. The Journal of Clinical Psychiatry, [epub ahead of print].
27. Kumar, M., G.N. Chandu and M.D. Shafiulla, 2006. Oral health status and treatment needs in institutionalized psychiatric patients: one year descriptive cross sectional study. Indian Journal of Dental Research, 17(4):171-177.
28. Ramon, T., A. Grinshpoon, S.P. Zusman and A. Weizman, 2003. Oral health and treatment needs of institutionalized chronic psychiatric patients in Israel. European Psychiatry,18(3):101-105.
29. Persson, K., B. Axtelius, B. Söderfeldt and M. Ostman, 2010. Oral health-related quality of life and dental status in an outpatient psychiatric population: a multivariate approach. International Journal of Mental Health Nursing, 19(1):62-70.
30. Hede, B. and P.E. Petersen, 1992. Self-assessment of dental health among Danish noninstitutionalized psychiatric patients. Special Care in Dentistry, 12(1):33-36.
31. Chu, K.Y., N.P. Yang, P. Chou, H.J. Chiu and L.Y. Chi, 2010. Factors associated with dental caries among institutionalized residents with schizophrenia in Taiwan: a cross-sectional study. BMC Public Health, 10:482.
32. Gowda, E.M., P.S. Bhat and M.M. Swamy, 2007. Dental health requirements for psychiatric patients. Medical Journal Armed Forces of India, 63:328-330.
33. Lewis, S., R.G. Jagger and E. Treasure, 2001. The oral health of psychiatric in-patients in South Wales. Special Care in Dentistry, 21(5):182-186.
34. Rekha, R., S.S. Hiremath and S. Bharath, 2002. Oral health status and treatment requirements of hospitalized psychiatric patients in Bangalore city: a comparative study. Journal of Indian Society of Pedodontics and Preventive Dentistry, 20(2):63-67.
35. Callaghan, R.C., M.D. Boire, R.G. Lazo, K. McKenzie and T. Cohn, 2009. Schizophrenia and the incidence of cardiovascular morbidity: a population-based longitudinal study in Ontario,

Canada. Schizophrenia Research, 115(2-3):325-332.
36. Hennekens, C.H., A.R. Hennekens, D. Hollar and D.E. Casey, 2005. Schizophrenia and increased risks of cardiovascular disease. American Heart Journal, 150(6):1115-1121.
37. Yolken, R.H. and E.F. Torrey, 2008. Are some cases of psychosis caused by microbial agents? A review of the evidence. Molecular Psychiatry, 13(5):470-479.
38. World Health Organization, 1992. The ICD-10 Classification of Mental and Behavioural Disorders. Geneva: World Health Organization.
39. Kay, S.R., A. Fiszbein and L.A. Opler, 1987. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophrenia Bulletin, 13(2): 261–276.
40. Nelson, K.E., R.D. Fleischmann, R.T. DeBoy, I.T. Paulsen, D.E. Fouts, J.A. Eisen, 2003. Complete genome sequence of the oral pathogenic Bacterium *porphyromonas gingivalis* strain W83. The Journal of Bacteriology, 185(18):5591-601.
41. Gu, F., Y. Li, C. Zhou, D.T. Wong, C.M. Ho, F. Qi and W. Shi, 2009. Bacterial 16S rRNA/rDNA Profiling in the Liquid Phase of Human Saliva. The Open Dentistry Journal, 3:80-84.
42. Layton, A., L. McKay, D. Williams, V. Garrett, R. Gentry and G. Sayler, 2006. Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. Applied and Environmental Microbiology, 72(6):4214-4224.
43. Lyons, S.R., A.L. Griffen and E.J. Leys., 2000. Quantitative real-time PCR for *Porphyromonas gingivalis* and total bacteria. Journal of Clinical Microbiology, 38(6):2362-2365.
44. Borrell, L.N., B.A. Burt, H.W. Neighbors and G.W. Taylor, 2008. Social factors and periodontitis in an older population. American Journal of Public Health, 98(9 Suppl):S95-101.
45. Jones, B.J., B.J[3rd.] Gallagher, A.M. Pisa and J.AJr. McFalls, 2008. Social class, family history and type of schizophrenia. Psychiatry Research, 159 (1-2):127-132.
46. Seymour, C.W., T.W. Iwashyna, C.R. Cooke, C.L. Hough and G.S. Martin, 2010. Marital status and the epidemiology and outcomes of sepsis. Chest, 137(6):1289-1296.
47. Shchipkova, A.Y., H.N. Nagaraja and P.S. Kumar, 2010. Subgingival microbial profiles of smokers with periodontitis. The Journal of Dentistry Research, 89(11):1247-1253.
48. AI-Otaibi, M., M. Zimmerman and B. Angmar-Månsson, 2003. Prevailing oral hygiene practices among urban Saudi Arabians in relation to age, gender and socioeconomic background. Acta odontologica Scandinavica, 61:212-216.
49. Asadi, S.G. and Z.G. Asadi, 1997. Chewing sticks and the oral hygiene habit of the adult Pakistani population. International Dental Journal, 47: 275-278.
50. Darout, I.A., J.M. Albandar and N. Skaug, 2000. Periodontal status of adult Sudanese habitual users of miswak chewing sticks or toothbrushes. Acta Odontologica Scandinavica, 58: 25-30.
51. Nuechterlein, K.H., M.F. Green and R.S. Kern, 2009. Schizophrenia as a Cognitive Disorder: Recent Approaches to Identifying its Core Cognitive Components to Aid Treatment Development. Advances in Schizophrenia Research, 4:267-282.
52. Kaye, E.K., A. Valencia, N. Baba, A[3rd]. Spiro, T. Dietrich and R.I. Garcia, 2010. Tooth loss and periodontal disease predict poor cognitive function in older men. The Journal of the American Geriatrics Society, 58(4):713-718.
53. Noble, J.M., L.N. Borrell, P.N. Papapanou, M.S. Elkind, N. Scarmeas and C.B. Wright, 2009. Periodontitis is associated with cognitive impairment among older adults: analysis of NHANES-III. Journal of Neurology, Neurosurgery, and Psychiatry, 80(11):1206-1211.

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