

# Periodontal disease and coronary heart disease: an epidemiological and microbiological study

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## SUMMARY

**Aims:** This is an investigation on the association between periodontal disease and an increased risk of coronary heart disease; the main hypothesis is that periodontal infections may increase the systemic inflammatory burden of the host above a threshold that may favour the atherogenic processes.

**Materials and Methods:** Case-control study with 27 cases, cardiologically affected, and 15 healthy controls. Patients underwent a complete periodontal probing. Periodontal conditions were compared between cases and controls to assess the mentioned association and to search for periodontal conditions related to the increased coronary risk. The presence and prevalence of periodontal pathogens was assessed in crevicular fluid samples.

**Results:** The overall periodontal conditions resulted worse in the test group. In particular periodontal conditions such as the presence of deep pockets (probing depth >6 mm) and the loss of more than 12 teeth might represent indicators of a strongly increased risk of cardiological disease and microbiological investigations confirmed these findings; *Prevotella gingivalis* was the most common bacteria.

**Conclusion:** This study supports the existence of an epidemiologic association between periodontal disease and coronary heart disease and confirms previous data present in the literature. Two periodontal parameters, deep pockets and number of missing teeth, seem to be important risk factors for cardiovascular diseases.

**KEY WORDS:** Periodontal disease, Periodontal pathogens, Coronary heart disease, Risk factor, Association

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## INTRODUCTION

Periodontitis is the most common mouth disease in the adult population of the industrialized countries (AAP 1996, NIDR 1987) and is also the primary cause of tooth loss among the same populations (Flemming, 1999). The aetiology of periodontitis is infectious, and a poor level of oral hygiene and the accumulation of dental plaque is the cause of the manifestation of the disease.

The pathogenesis of periodontitis is complex and multifactorial; inflammation and host related factors are of crucial importance in the development and severity of the disease (De Nardin, 2001). In particular some genetically determined features of the immune response of the host such as the capability of macrophages to limit the growth of the bacterial populations, the production of inflammatory cytokines, their presence and concentration in the periodontal tissues and in the crevicular fluid, are determinant factors in the development of the signs and symptoms of the disease such as attachment and alveolar bone loss and destruction/resorption (Jandinski *et al.*, 1991; Stashenko *et al.*, 1991; Tatakis, 1993; Preiss and Meyle, 1994).

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Many bacteria are thought to be involved in the pathogenesis but a restricted group has proved to be the cause of the disease; this list includes *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia*.

Cardiovascular disease and in particular atherosclerosis is the first cause of death among the population of the industrialized countries, especially adult males over 50. In the 1990's it was responsible for 50% of all deaths in these countries with increasing incidence and prevalence (Ross 1993). Many of the risk factors for atherosclerosis are well known but there is a large number of cases with atherosclerosis without any of the known risk factors.

During the nineties a new theory (Ross, 1993) to explain the formation and growing of the atherosclerotic plaque was proposed on the basis of a subclinical but chronic inflammatory state. Altered levels of inflammatory mediators in the patient could be the cause either of endothelial damage, which is the first event of the atherosclerotic plaque formation (Woodward *et al.*, 1999; Ridker *et al.*, 2000; Lowe 2001), or enhance the speed of tissue damage in the vascular wall and the restriction of the lumen of the vessel via stimulation of endothelium, macrophages, smooth muscle cells to proliferate, and progressively to the restriction of the lumen of the involved vessel (Ross 1993). When the plaque is sufficiently thick it becomes vascularized and undergoes processes of fissuration, haemorrhage and ulceration that leads to the complete obstruction of the lumen of the vessel or the detachment of critical size thrombus capable of occluding smaller arteries (Davies and Thomas, 1984); in this terminal phase of the pathology it is possible to see an increased level of cytokines and other mediators.

By the end of the last decade many authors pointed out that periodontal disease could be a possible risk factor for atherosclerosis (Beck *et al.*, 1996; Offenbacher *et al.*, 1999; Grau *et al.*, 2004). Periodontitis is a chronic infection that enhances the production of certain types of cytokines such as PCR, IL1, IL 6 TGF $\beta$  (D'Aiuto *et al.*, 2004a). This alteration lasts for long periods of time since many patients do not take care of this problem or even worse are not aware of being affected until the problem is very severe. Many studies

revealed a higher prevalence of periodontitis in atherosclerotic patients, but no one is capable of explaining this finding in terms of cause effect or simple coexistence of the two conditions (Mattila *et al.*, 1989 and 1993; De Stefano *et al.*, 1993; Joshipura *et al.*, 1996; Genco *et al.*, 1999; Morrison *et al.*, 1999). On the other hand a few studies failed to demonstrate such an association (Hujoel *et al.*, 2000; Mattila *et al.*, 2000; Howell *et al.*, 2001). Despite the large number of studies present in the literature the exact mechanisms involved in this association are not fully understood. It is worth emphasizing that it is very difficult to demonstrate or not, a cause-effect relationship between two chronic conditions because of the long period of time necessary and because periodontitis can play a role in one of the many phases of the formation, growth and rupture of the plaque (Genco *et al.*, 2002) and it is impossible to understand when this presumed role acts actively (Wick *et al.*, 1995; Lagrand *et al.*, 1999; Deliargys *et al.*, 2000). Some literature reviews have been published on this topic, but definitive conclusions cannot be drawn (Beck and Offenbacher 1998 and 2001). Besides this, both the conditions share many other risk factors, in particular smoking, (Spiekermann *et al.*, 2003) that complicate the researcher's investigations and leads to results which are difficult to interpret because of these confounding factors.

Most studies in the literature are on American, Japanese or north European populations, while few have addressed southern European Mediterranean populations.

Aim of our study was to evaluate the mentioned association in the Italian population, in which chronic diseases will become a major medical problem in the next few years as the average age of this population is rapidly increasing especially in Genoa.

## MATERIALS AND METHODS

Patients of the test group were recruited among those who received a diagnosis of myocardial infarction or angina pectoris in a period of time going from 2002 and 2004. All the subjects underwent an angiographic examination of the coronary arteries which always revealed stenosis of at least one coronary vessel. The diagnosis of

angina pectoris was given to patients with multiple episodes of thoracic pain lasting more than 15 minutes arising under physical efforts or spontaneously, but declining with rest or administration of nitro-glycerine. The diagnosis of acute myocardial infarction was given electrocardiographically and /or by enzymatic measurements (Beck *et al.*, 1996). The medical history of all patients was investigated as well as the cardiovascular risk factors such as hypertension, dyslipidemia (either hypercholesterolemia or hypertriglyceridemia) and smoking habits. All these factors were not evaluated quantitatively but only as present or absent in a dichotomic fashion. With these criteria a group of 26 men and 1 woman aged 40 to 70 years was recruited for the periodontal examination as a test group.

The control group was recruited from the population of the same geographic area in order to meet the characteristics of the test group from the socioeconomic status, sex and age. The group was then composed of 15 men and 1 woman from 45 to 65 years of age. All these subjects underwent medical check-ups in the period 2002-2004 able to exclude the presence of angina pectoris or other manifestations of coronary atherosclerosis. The medical history and information on cardiovascular risk factors were collected in the same way as for the test group.

Both test and control groups underwent a complete periodontal probing to avoid problems in diagnosis typical of the simplified methods such as Community Periodontal Treatment Index Needs (CPTIN), mainly used in previous studies (AAP 1996, NIDR 1987, Flemming 1999). A standard round edge millimetrated 15 mm length probe (CPC 15 UNC) was used to collect data. The probing was performed on 6 sites per tooth 3 buccal and 3 palatal/lingual. Every tooth present in the mouth of each patient was probed even though it lacked a clinical crown; teeth with a bad prognosis were also probed.

With this visit the following information was collected: number of missing teeth (MT), bleeding on probing index (BOP) percentage, probing depth (PD) 6 sites per tooth, Clinical Attachment Level (CAL) 6 sites per tooth. Then the mean PD for each patient was calculated, as well as the number of sites with a PD >6mm and the number of sites with 4 mm <PD <6 mm for each patient; the mean CAL for each patient. For each

patient the site with the highest PD score was selected for the subgingival plaque sampling. The microbiological samples were collected with a smooth needle for endodontic rinsing, inserted in the bottom of the pocket after having thoroughly cleaned the chosen site from the supragingival plaque with a toothbrush and isolated with cotton rolls to prevent saliva contamination. Specimens were then sent in appropriate containers to the microbiological laboratory. Finally, samples were plated on rich and selective media including Brucella blood agar, kanamycin-vancomycin lacked blood agar. Plates were incubated anaerobically and in an atmosphere containing 5% CO<sub>2</sub> for 5 days. Isolated pathogens were identified to the species level by the RapID ANA II system (Remel, USA). *Tannerella forsythia* was detected using PCR and confirmed by restriction analysis as described by Narayanan *et al.*, (2005).

Student's T test was used to compare groups: the average age of the two groups, the known and investigated cardiovascular risk factors, and periodontal parameters such as the mean PD, the mean CAL and the BOP. Then the presence and percentage of sites with PD >6 mm, sites with 4 <PD <6 was calculated for each subject and data were compared between groups.

A non-parametric dichotomic evaluation was performed for: number of sites with PD >6 mm; CAL average score; number of missing teeth. For the first parameter the dichotomization was carried out in two ways:

- a) giving score 1 to all the patients with at least 3 sites probing 6 mm or more and 0 to the others;
- b) giving score 1 to all the patients with 1 or more sites probing 6mm or more and 0 to the others.

For the CAL the dichotomization was made on the average value, if it was CAL (average score) > 3.5mm the score was 1 in all other cases 0. All the patients with 12 or more missing teeth received score 1 and all the others score 0. The number of missing teeth was calculated from the base of 28, wisdom teeth did not account for this evaluation. For all this non-parametric data the Fischer test was used to compare the two groups. For the microbiological analysis the patients were searched for periodontal pathogens as mentioned above. After this evaluation the results were sum-

marized in a dichotomic index giving score 1 to patients harbouring one or more periodontal pathogen and 0 to the others and again the Fisher test was performed to compare the two groups.

## RESULTS

The average age of the two groups was, as expected, not statistically different with a value for the test group of 57.7 and for the control of 55.1 ( $p$ -value 0.217).

The only cardiovascular risk factor found with a statistically significant difference was the incidence of dyslipidemia, higher in the test group. For all the other risk factors the distribution was similar in both groups (Table 1).

Among the periodontal parameters a significant difference was found only for the number of missing teeth (Table 2), and the number of sites probing more than 6mm, as reported in Table 3. This last index was significant however it was investigated: average number per patient, percentage, first and second dichotomization.

The average CAL was not found to be statistically significantly different in our sample. Table 3 sum-

marises the results observed and the  $p$  values obtained in the statistical analysis.

Microbiological analysis data are summarized in Table 4. Interestingly many of the plaque samples harvested from subjects of the case group harboured at least one periodontal pathogen species (89.4%) compared to the incidence in the control group which was much lower (46.6%). The distribution of the species is summarized in Table 4; above all the Fisher test revealed a statistically significant difference in the two groups for the incidence of the periodontal pathogens, but none of the investigated species was significantly more present than other species.

## DISCUSSION

The present findings confirm those reported by others and indicate a possible relation between periodontal disease and cardiovascular atherosclerosis (De Stefano *et al.*, 1993; Mattila *et al.*, 1989, 1993 and 2000; Joshipura *et al.*, 1996; Genco *et al.*, 1999; Morrison *et al.*, 1999).

In particular, all the periodontal parameters are worse in the test than in the control group, even

TABLE 1 - Distribution of cardiovascular risk factors in cases (cardiopathic patients) and controls (healthy patients).

Underlying disease (present)	Patients		P
	Cardiopathic (test group)	Non cardiopathic (control group)	
Hypertension	11 (40.7%)	3 (18.7%)	0.144
Diabetes	1 (3.7 %)	2 (12.5%)	0.285
Dyslipidemia	22 (81.5%)	4 (25%)	< 0.001
Smoking	13 (48.1%)	7 (43.7%)	0.786

TABLE 2 - Average values of periodontal parameters in test and control group.

	BoP %	PD mm	CAL mm	MT n°
Cardiopathic (test)	32.1	2.93	3.45	14.6
Non cardiopathic (control)	35	2.81	3.12	7.8
P	0.572	0.426	0.145	0.045

BoP = Bleeding on Probing; PD = Probing Depth; CAL = Clinical Attachment Level; MT = Missing Teeth; Mm = millimetres

TABLE 3 - Analysis of periodontal parameters in test and control group.

	CAL		MT		Sites 4 mm<= PD <= 6 mm		Sites PD >6 mm					
					Avarage values		Avarage values		Dichotomic index A		Dichotomic index B	
	Rank 0	Rank 1	Rank 0	Rank 1	N <sup>o</sup> *	%§	N <sup>o</sup> *	%§	Rank 0	Rank 1	Rank 0	Rank 1
Test	10	9	16	11	35.11	24.26	3.79	2.49	10	9	2	17
Control	13	2	15	1	31.47	20.45	1.20	0.79	14	1	7	8
P	0.064		0.017		0.486	0.288	0.049	0.036	0.020		0.025	

CAL (Clinical Attachment Level)  
 - rank 0: patients with average CAL <3.5 mm  
 - rank 1: patients with average CAL >3.5 mm  
 MT: ( Missing Teeth)  
 - rank 0: patients with <12 MT  
 - rank 1: patients with >12 MT  
 \*Number of sites per patients; §% of sites on the total number of sites probed per patient

Dichotomic index A  
 - rank 0: patients with 3 or more sites with PD >6 mm  
 - rank 1: patients with less than 3 sites with PD >6 mm  
 Dichotomic index B  
 rank 0: patients with 1 or more sites with PD >6 mm  
 rank 1: patients with no sites with PD >6 mm

though for a few of these the statistical significance of these differences is not reached. The most interesting parameters are the presence of sites with PD >6 mm and the average CAL values. With regard to the presence of deep pock-

ets the difference is statistically different whatever the investigation: absolute number, percentage of deep pockets on the total of sites probed, ranked dichotomic non-parametric transformation in both ways it was performed.

TABLE 4 - Distribution of the investigated periodontal pathogens in both test and control groups.

	Cardiopathic (test)	Non Cardiopathic (control)
<i>Porphyromonas gingivalis</i>	9 (47.4%)	1 ( 6.6%)
<i>Prevotella intermedia</i>	4 (21%)	0
<i>Prevotella buccae</i>	4 (21%)	1 (6.6%)
<i>Prevotella oralis</i>	4 (21%)	3 (20%)
<i>Tannerella forsythia</i>	2 (10.5%)	0
<i>Fusobacterium nucleatum</i>	4 (21%)	2 (13.3 %)
<i>Capnocytophaga sputigena</i>	1 (5.3%)	0
<i>Bifidobacterium</i>	3 (15.8%)	0
<i>Streptococcus constellatus</i>	4 (21%)	7 (46.6%)
Subjects with at least one pathogen	17	7
Subjects without pathogens	2	8
P-value	0.01	

This kind of result strongly indicates the possibility that having deep pockets is a risk factor of developing and/or worsening a coronary atherosclerosis. In fact only deep pockets can harbour significant colonies of periopathogens (Kuramitsu *et al.*, 2001) and can stimulate a significant inflammatory response able to influence systemic health such as the development and/or worsening of cardiovascular atherosclerosis (Wick *et al.*, 1995; Deliargys *et al.*, 2000; Loos *et al.*, 2000; WU *et al.*, 2000; De Nardin, 2001; Noack *et al.*, 2001).

The bacterial species isolated in this study are in accordance with the literature (Haffajee *et al.*, 2004), with the exception of *Prevotella* genus, which was more frequent in our geographic area, as well as *S. constellatus*, an opportunistic pathogen collected from cardiopathic and non cardiopathic patients.

On the contrary the fact that the average PD is only moderately different in the two groups, without statistical significance can be explained by the fact that in the control group this result emerges from a condition of moderate but widespread increase in PD, whether in the test group there is the constant presence of some deep pockets and a few number of other sites with moderate PD. In fact in the analysis of the shallow pockets, 4 mm <PD <6 mm there was no difference between the two groups but there was a difference for deep pockets, PD >6 mm.

Regarding the CAL no statistical difference was found between the two groups, but the test group had a worse performance. Interestingly after ranking the CAL in a dichotomic way, as illustrated above, the difference between the two groups was still non significant but the p-value was 0.064, very close to significance. In our opinion this is correlated with the small size of our sample and with a larger group the value may have reached significance. This result is easily explained by the fact that a CAL loss without deep pockets is measured in recessions of the gingival margin. CAL is an "historic index" of periodontal conditions, it mirrors the effects of past, recent or present episodes of periodontal disease in the clinical history of the patient; so patients with an average CAL loss >3.5mm could have been exposed to periodontal disease for a long time. Periodontal disease is a chronic condition with episodes of recrudescence resulting in CAL

loss. Recent evidence (D'aiuto *et al.*, 2004) indicates that periodontal disease is also a chronic but subclinical inflammatory stimulus that can lead to systemic alterations of some haematic proteins such as PCR (Ebersole *et al.*, 1997). On the other hand there is evidence that the inflammatory state is a risk factor for developing atherosclerotic plaques (Ross, 1999; Libby *et al.*, 2002; Pearson *et al.*, 2003). So if a patient has an average CAL of 3.5 mm or more s/he must have been exposed to a long history periodontal disease and so a long period of time with possible alteration of important inflammatory markers able to promote atherogenesis.

The same explanation must be given for other findings emerging from the present results, regarding the number of missing teeth. The first cause of tooth loss in western countries is periodontitis and so we can assume that our patients with a lot of missing teeth have had a long history of periodontitis (Lowe 2001, Grau *et al.*, 2004). In our sample the number of missing teeth differed between the two groups with statistical significance. All these data point out that periodontitis can be seen as a cardiovascular risk factor, but that it must reach a certain severity to be able to have systemic implications. Very interestingly test group patients presented at least one deep pocket with a frequency significantly greater than that of controls, whereas the average PD did not differ between the two groups. This result finds a biologic explanation in the inflammatory hypothesis of atherosclerotic plaque formation (Ross 1993). In fact only in deep pockets was the bacterial load sufficient to give systemic responses.

The microbiological findings confirm the results of the analysis of the periodontal parameters as the presence of at least one periopathogen was statistically more frequent in the test group than in the controls. This is indirect confirmation of the worse periodontal status of those patients and suggests that harbouring one of these bacterial species for a long period of time can induce a dangerous systemic response (Dorn *et al.*, 1999; Genco *et al.*, 1999). On the other hand, any statement on which species is a possible risk factor can not be made, because the different species were found in different patients without any statistical difference. The interesting trend is that *P. gingivalis* was found in almost half of the test

group patients whereas there was not the same prevalence in the control group. This result is consistent with others present in the literature and with some pathogenic characteristics of this species that suggest the capability to induce a systemic inflammatory response (Desphende *et al.*, 1998; Geva *et al.*, 2000; Herzberg, 2001).

In conclusion, our findings confirm literature in the direction of a statistical association between periodontitis and atherosclerosis. In particular in this trial the presence of deep pockets appeared to be a strong risk factor for coronary heart disease. Our study is a case control study so, despite a strong association, we cannot make any statement of a possible cause-effect relationship between the two conditions. This must be clarified by other studies such as interventional studies or very long cohort prospective studies.

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