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Periodontitis and atherosclerosis: an observational study

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Background: Poor oral health has been related with cardiovascular diseases. Specifically, periodontitis has been implicated in the pathogenesis of atherosclerosis. Our aim was to evaluate the relationship between the degree of carotid atherosclerosis and severity of periodontitis in a patient cohort.

Methods: Fifty adult patients receiving carotid duplex scans in a cerebral hemodynamics lab were included in the study. Ultrasound protocol included measurement of carotid intima–media thickness (IMT), which is a marker of atherosclerosis, and characterization of any atherosclerotic plaques in the cervical common and internal carotid arteries. Patients were divided into two main groups: the test group had IMT $\geq$ 1 mm or the presence of any carotid atherosclerotic plaque, and the control group had IMT < 1 mm and absence of atherosclerotic plaques. Periodontal evaluation was performed in all the teeth and characterized according to the clinical attachment level, which between 1 mm and 2 mm was classified as slight, 3 mm and 4 mm as moderate and $\geq$5 mm as severe.

Results: The control group included 15 (30%), while the test group included 35 (70%) subjects. The most common diagnosis was severe periodontitis (40%); moderate and slight periodontitis were also frequent occurrences (32% and 28%, respectively). In the control group, 53.3% had slight, 33.3% had moderate and 13.3% had severe periodontitis. In the test group, those percentages were respectively 22.2%, 44.4% and 33.3%. Patients with atheroma plaque had the highest percentage of severe periodontitis (70.6%). More severe periodontitis was related to atherosclerosis ($P = 0.007$).

Conclusion: This study showed an association between periodontitis severity and carotid atherosclerosis, suggesting that periodontal disease might be a risk indicator for atherosclerotic disease.

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Worldwide, according to the World Health Organization (WHO), ischemic heart disease and stroke are responsible for more deaths (12.2% and 9.7%, respectively) than the cancers altogether. Owing to population aging and increase in vascular risk factor prevalence, it is estimated that the percentage of vascular deaths will reach 31.7% in 2030 (1), despite all the developments in prevention, diagnosis and treatment.

Vascular disease is mainly due to atherosclerosis, a condition in which an artery wall thickens because of the accumulation of fatty materials such as cholesterol, associated with cellular changes and chronic inflammation in the vessel walls. After an increase in the thickness of the intima–media (IMT) layers of the wall, this can progress to the development of an atherosclerotic plaque, with progressive narrowing of the lumen. Clinical

consequences might be an ischemic accident, either by distal embolism of a plaque fragment or by progression of the luminal stenosis until occlusion of the artery (2–6).

Since the late 1980s, several papers and studies have linked poor oral health with cardiovascular diseases (CVD) (7). Periodontal disease, and specifically periodontitis, has been assumed as a possible factor implicated in the etiopathogenesis of atherosclerosis (2,4). A possible link between the severity of periodontitis and IMT of the carotid artery arose in 2001, when a positive association between them was found (8). In the following year, an association between bone loss and increased calcium depostis in the carotid artery wall was described (9). In a pilot study, Pussinen *et al*. also evaluated a possible relationship between periodontitis and carotid IMT (10). Later, Franek *et al*., studying a female group of patients, suggested that periodontitis was associated with the development of atherosclerotic injuries in women (11). In 2008, other authors evaluating a group of patients aged under 40 years old and without any systemic pathologies, noticed that severe periodontitis was linked with subclinical atherosclerosis (12).

Periodontal disease is site specific, which continuously fluctuates with exacerbation and remission periods (13), and its progession is facilitated by the morphological characteristics of the affected tissues. The clinical consequences of this pathology, as well as its progression and severity, depend on the host's inflammatory and immune response to the specific bacteria that become part of the oral biofilm (14).

Periodontitis requires the presence of oral bacteria, and some of these have been identified in atheromatous plaques (1,2) Furthermore, specific antibodies, reacting to periodontal bacteria and pro-inflammatory proteins, have been isolated from patients affected by strokes (2,15–18).

In the literature, some theories have been postulated regarding a possible relation between periodontitis severity and presence of atherosclerosis, although information is still missing, as a positive relationship was only determined for the most severe forms of oral pathology (8,12). Therefore, the objective of this study was to evaluate whether there is a correlation between the degree of atherosclerosis and periodontitis severity.

Material and methods

The study was conducted in Hospital São João, a tertiary university hospital in Porto, Portugal. Institutional ethical approval was obtained from *Comissão de Ética do Hospital de São João*. Subjects signed written informed consent, after the purpose and procedures of the study were carefully explained to each patient.

Fifty adult patients that consecutively received carotid duplex scans in the cerebral hemodynamics laboratory of the hospital from June to September 2009 were included in the study, as long as they matched the inclusion and exclusion criteria.

A medical history was collected, including smoking habits, pregnancy status, information concerning systemic pathologies and type of medication (including anti-inflammatory drugs).

Inclusion criteria were age $\geq$ 18 years old, having no contraindications to perform the oral examination, accepting the protocol and having signed the informed consent.

Exclusion was applied to edentulous patients, pregnant patients, patients needing infectious endocarditis prophylaxis before the periodontal examination, or any other reason that would prevent the periodontal examination.

Carotid ultrasound protocol

The carotid evaluation was performed with a high-resolution echography device, the Philips HDI 5000 ultrasound imaging platform (Philips Healthcare, Andover, MA, USA), using a linear probe (frequency 5–12 MHz). All the images were acquired by the same neurosonology expert, before the periodontal examination, obeying to the international consensus recommendations published in 2007 (19). The IMT was measured along a minimum of 10 mm length of the distal common carotid artery and proximal internal carotid artery, in the longitudinal plane, perpendicular to the ultrasound beam, revealing both near and far arterial walls. After several measurements with the manual method (IMT measurements were done on the screen of the sonography device during the sonographic exam and supported by a digital archiving system), the thicker point was selected, to increase the reliability and reproducibility of the technique. In addition to these IMT determinations, the presence of atherosclerotic plaques in the common and/or internal carotids was recorded. According to the Mannheim carotid IMT consensus (19), IMT was defined as a double-line pattern visualized by echotomography on both walls of the artery in a longitudinal image, being formed by two parallel lines, which consist of the leading edges of two anatomical boundaries: the lumen–intima and media–adventitia interfaces. Plaque was defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness $>$ 1.5 mm as measured from the media–adventitia interface to the intima–lumen interface.

Patients were divided into two main groups, according to carotid ultrasound imaging: the test group had IMT $\geq$ 1 mm or the presence of any carotid atherosclerotic plaque, and the control group had IMT $<$ 1 mm and absence of atherosclerotic plaques.

Periodontal evaluation protocol

The periodontal examination was performed in all teeth (six points per tooth) with a manual periodontal North Carolina probe (Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). The same investigator (MP) previously calibrated and evaluated the following clinical variables: plaque index (dichotomic visual plaque index by J. Ainamo) (20); bleeding on probing; probing depth (PD); gingival recession; clinical attachment level (CAL).

The periodontal diagnosis was determined according to Armitage criteria (21). The severity of each clinical case was determined according to CAL. CAL between 1 mm and 2 mm was classified as slight, 3 mm and 4 mm as moderate and ≥ 5 mm as severe.

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences software, SPSS® vs.17.0 (SPSS Inc., IL, Chicago, USA), considering a significance level of 0.05 ($\alpha = 0.05$).

The Kruskal–Wallis test was used to compare central tendency measures (median) in more than two groups, when the normality of distribution and homogeneity of variances were not observed (by the Shapiro–Wilk test; $n \leq 50$).

The Mann–Whitney test was used to compare the central tendency measures of two independent groups. Association between categorical variables was assessed using the chi-squared or the Fisher test.

In multivariate analysis, the independent effect of significant variables or covariates ($P < 0.05$) on periodontal disease and IMT was assessed using binary logistic multivariate regression analysis (Wald backward stepwise method, $P = 0.05$ for covariate inclusion and 0.1 for exclusion). Age was dichotomized (< 50 years; $\geq$ 50 years) for univariate analysis, but was used as a continuous variable in multivariate analysis for ages $\geq$ 50 years (where age the group < 50 years was considered as the reference group).

Results

Demographic and ultrasound data

The characterization of the study population is presented in Table 1.

The sample was composed of 50 individuals (25 men and 25 women) with an average age of 56.1 (± 14.8) years.

Fifteen (30%) of the individuals had an IMT < 1 mm (control group) and 35 (70%) had an IMT $\geq$ 1 mm and/or atheroma plaque (test group).

Table 1. Demographic and clinical description of patients in study. The most relevant statistics for continuous variables are presented

	Control group	Test group		
	IMT < 1 mm ($n = 15$)	IMT $\geq$ 1 mm ($n = 18$)	Atheroma plaque ($n = 17$)	*P*-value
All	15 (30%)	18 (36%)	17 (34%)	
Gender				
Male	4 (26.7%)	9 (50%)	12 (70.6%)	0.046*
Female	11 (73.3%)	9 (50%)	5 (29.4%)	
Age (years)				
Average (± SD)	40.8 (± 11.9)	62.9 (± 10.4)	62.5 (± 10.9)	< 0.001**
Me (P_{25}–P_{75})	40^{b} (31–52)	63^{a} (58–71)	64^{a} (55–68.5)	
Min–max	19–56	34–77	38–80	
Age				
< 50 years	11 (73.3%)	2 (11.1%)	32 (11.8%)	<0.001*
$\geq$ 50 years	4 (26.7%)	16 (88.9%)	15 (88.2%)	
Tobacco				
No	14 (93.3%)	18 (100%)	12 (70.6%)	0.021***
Yes	1 (6.7%)	(–)	5 (29.4%)	

IMT, intima–media thickness; Me, median; P25 and P75, 25th and 75th percentiles; SD, standard deviation.
*Chi-squared independency test.
**Kruskal–Wallis' test.
***Fisher's test (IMT $\geq$ 1 mm and atheroma plaque joined as a homogeneous group).
Different superscript letters show significant differences in the median age value within the respective groups.

The study included significantly more women (73.3%) within the control group. For the test group, differences between genders only occurred in patients who already had atheroma plaque (men 70.6%, $P < 0.05$).

The average age of the control group (40.8 ± 11.9) was significantly different from the test group [(IMT $\geq$ 1 mm (62.9 ± 10.4), atheroma plaque (62.5 ± 10.9)] and, namely, patients over 50 years old were more represented in the test group ($P < 0.001$).

The overall percentage of smokers was 12%. They were more prevalent in the test group (29.4% vs. 6.7%, $P < 0.05$), and all the smokers in this group had atheroma plaque.

Regarding previous vascular events, 4% of the sample had previous acute myocardial infarction, 32% had a stroke and 24% had diabetes, with no significant differences between the study groups (Table 2).

The multivariate analysis showed that IMT progression was significantly associated with an increase in patient's age: each year the probability of having an IMT $\geq$ 1 mm increases 1.2 times and the probability of having an atheroma plaque approximately 1.3 times (Table 3).

Periodontal data

The periodontal data are presented in Table 2.

For all patients ($n = 50$) the mean percentage of sites with PD $\geq$ 4 mm was 35.5% (data not shown in Table 2).

Regarding the extent of PD, the majority of sites occurred in the test group: 33.9% for IMT $\geq$ 1 mm without plaque and 47.1% for the atheroma plaque group (Table 2). In the control group the corresponding value was 24.3%; the differences were statistically significant among groups ($P < 0.05$). The percentage of PD < 4 mm was higher in the control group ($P < 0.05$).

The plaque index was 61.4% within the control group, and 70.8% and 70.3% respectively with and without atheroma plaque within the test

Table 2. Clinical description of patients in the study. The most relevant statistics for continuous variables are presented

	Control group	Test group		
	IMT < 1 mm (n = 15)	IMT ≥ 1 mm (n = 18)	Atheroma plaque (n = 17)	P-value
PD				
< 4 mm	11 (73.3%)	12 (66.7%)	5 (29.4%)	0.023*
≥4 mm	4 (26.7%)	6 (33.3%)	12 (70.6%)	
n sites PD < 4				
Average (± SD)	105.9 (± 57.2)	89.2 (± 47.6)	58.5 (± 38.1)	0.011**
Me (P_{25}–P_{75})	127[a] (33–151)	107[b] (67.8–123.5)	50[c] (27–84)	
Min–max	1–167	0–136	0–136	
n sites PD ≥ 4				
Average (± SD)	35.4 (± 53.1)	33.8 (± 33.3)	51.1 (± 33.3)	0.042**
Me (P_{25}–P_{75})	11[b] (2–38)	25[b] (11.5–43.5)	52[a] (20.5–79)	
Min–max	0–167	0–138	5–107	
Extent PD (%) ≥ 4				
Average (± SD)	24.3 (± 32.9)	33.9 (± 34.3)	47.1 (± 28.1)	0.032**
Me (P_{25}–P_{75})	9[b] (1.2–29.2)	22.2[ab] (8.1–48.5)	50[a] (23.1–66.9)	
Min–max	0–99.4	0–100	5–100	
Plaque Index (%)				
Average (± SD)	61.4 (±22.4)	70.3 (±27.8)	70.8 (±32.2)	0.453**
Me (P_{25}–P_{75})	67.5 (46.1–75)	73.4 (50.9–98.4)	80.7 (49.4–100)	
Min–max	13.3–100	10–100	4.6–100	
Plaque index (%)				
IP < 15	1 (6.7%)	1 (5.6%)	1 (5.9%)	0.991***
IP ≥ 15	14 (93.3%)	17 (94.4%)	16 (94.1%)	
Bleeding on probing (%)				
Average (± SD)	35.7 (± 26.8)	43.4 (± 33.2)	31.6 (± 25.4)	0.610**
Me (P_{25}–P_{75})	41 (10.7–61.1)	26.6 (20.7–67.5)	27.5 (10–42.3)	
Min–max	0–75	0–100	0–90	
Periodontitis				
Slight	8 (53.3%)	4 (22.2%)	2 (11.8%)	0.007*
Moderate	5 (33.3%)	8 (44.4%)	3 (17.6%)	
Severe	2 (13.3%)	6 (33.3%)	12 (70.6%)	
Myocardial infarction				
No	15 (100%)	17 (94.4%)	16 (94.1%)	0.639***
Yes	– (–)	1 (5.6%)	1 (5.9%)	
Stroke				
No	11 (73.3%)	10 (55.6%)	13 (76.5%)	0.361*
Yes	4 (26.7%)	8 (44.4%)	4 (23.5%)	
Diabetes status				
No	14 (93.3%)	14 (77.8%)	10 (58.8%)	0.072*
Yes	1 (6.7%)	4 (22.2%)	7 (41.2%)	

IMT, intima–media thickness; PD, probe depth; Me, median; P25 and P75, 25th and 75th percentiles; SD, standard deviation.
*Chi-squared independency test.
**Kruskal–Wallis test.
***Fisher test.
Different superscript letter show significant differences in the median age value within the respective groups

group. No statistically significant differences were found among groups.

Bleeding on probing did not show statistically significant differences per group ($P > 0.05$): 35.7% in the control group; 43.4% in the IMT ≥ 1 mm group; and 31.6% in the atheroma plaque group.

All patients showed periodontitis, although with different degrees of severity. As Table 1 shows, the most common diagnosis was severe periodontitis (40%); moderate and slight periodontitis were also frequent occurrences at 32% and 28%, respectively.

Through a chi-squared test a significant association between periodontitis severity and the groups was detected ($P = 0.007$). In the IMT < 1 mm control group 53.3% had slight periodontitis, 33.3% had moderate periodontitis and 13.3% had severe periodontitis. In the IMT ≥ 1 mm group without plaque 22.2% of the patients presented slight periodontitis, 44.4% moderate periodontitis and 33.3% severe periodontitis. The atheroma plaque group revealed 11.8% with slight periodontitis, 17.6% moderate periodontitis and 70.6% severe periodontitis. In patients with previous stroke, more severe forms of periodontitis were linked to an IMT increase and atheroma plaque formation ($P < 0.001$).

Results from the multivariate analysis (Table 4) revealed that age was the only risk factor observed that was significantly associated with moderate periodontitis (OR = 1.09). On the other hand, three factors remained independently and positively associated with severe periodontitis: age, male gender and plaque index (age: OR = 1.10, OR calculated for increments of 1 year for ages ≥ 50; male gender: OR = 14.3; and plaque index: OR = 1.05, OR calculated for increments of 1%).

Discussion

The present study has shown an association between periodontitis severity and carotid atherosclerotic disease.

Chronic inflammation plays a fundamental role in atherogenesis, and chronic infection due to several agents such as lymphotoxin-alpha, has been postulated as a potential cause for this chronic inflammation (22). In this context several papers emerged, positively relating periodontitis with atherosclerosis-induced diseases, sup-

Table 3. Multivariate logistic regression for risk factors forecasting associated with the intima–media thickness (IMT)

		IMT > 1 mm			Atherome plaque		
	IMT < 1 mm	*P*-value	OR	IC 95%	*P*-value	OR	IC 95%
Age (year)	1	0.004	1.20	1.06–1.36	0.010	1.28	1.06–1.54
Smokers	1	–	–	–	0.063	32.65	0.82–1295.13[a]

[a]Excessively wide range due to the small sample of the smokers' group.
Variables included on the first step: gender, age (continuous for ages $\geq$ 50 years), plaque index, bleeding on probe, periodontal disease, acute myocardial infarction, stroke, high blood pressure, cholesterol, diabetes, anticoagulants, aspirin and smokers.

Table 4. Multivariate logistic regression for risk factors forecasting associated with periodontal disease

		Moderate periodontitis			Severe periodontitis		
	Slight periodontitis	*P*-value	OR	IC 95%	*P*-value	OR	IC 95%
Age (years)	1	0.014	1.09	1.02–1.16	0.031	1.10	1.01–1.20
Male	1	–	–	–	0.035	14.35	1.20–171.14
Plaque index	1	–	–	–	0.023	1.05	1.01–1.10

Variables included on the first step: gender, age (continuous for age $\geq$ 50 years), plaque index, bleeding on probe, periodontal disease, diabetes and smokers.

porting the theory that proinflammatory mediators are responsible for this association (3,17). Other explanations are related to the autoimmune response to the periodontal bacteria and proteins involved in atherosclerosis-induced pathologies (15,16,18), as well as with bacterial invasion, which affects platelets, endothelial cells and macrophages (23–25). Our study does not allow us to confirm any of these theories, because it did not include any microbiological or immunological analysis. However, as about 70% of patients with atheroma plaque had severe periodontitis, it suggests at least an association between the two pathologies. This group had the highest percentage of severe periodontitis, meaning that more severe periodontitis levels were related to more advanced atherosclerosis.

Within the test group, age and sex were found to have a confounding effect. Although it has no interaction effect on periodontal disease, neither on having or not atheroma plaque. Diabetes status had neither confounding nor interaction effect between both variables.

Numerous studies indicate a positive association between oral cavity pathologies (i.e. periodontal disease) and cardiovascular pathologies, where atherosclerosis assumes an important role. Tonetti stated that severe generalized periodontitis causes systemic inflammation and endothelial dysfunction (26). Nonetheless, the number of studies exploring a possible connection between periodontitis severity and IMT of the carotid artery are limited, confined to Beck *et al.* and Cairo *et al.* studies (8,12).

The thickening of the arterial intima–media layers can be a preliminary step in atheroma plaque formation. The underlying chronic inflammatory process can increase plaque instability, with rupture and thrombus adherence, conducting to eventually dramatic episodes such as strokes. Therefore, IMT acquires importance as an atherosclerosis marker, and correlates with stroke risk factors. Using methodology in accordance with the Mannheim Carotid Intima-Media Thickness Consensus (19) our population with IMT $\geq$ 1 mm and/or atheroma plaque also showed a higher vascular risk, as they were older, predominantly male and with more smoking habits.

Concerning the periodontal condition, this study suggested an association between the most severe forms of periodontitis and an increase of IMT. Franek *et al.* also found an association between periodontitis and atherosclerosis development, although they have not made an association between the increases in the severity of both pathologies (11). Although Beck *et al.* and Cairo *et al.* reported that presence of atherosclerosis and periodontal pathology severity were related, the first one was not subdivided (8,12). Therefore, this study seems to be the first one attempting to correlate between distinct levels of periodontal and atherosclerotic pathology, revealing a progressive increase in both pathologies.

Comparing the test and control groups, we observed statistically significant differences, not only for periodontal disease severity, but also for age and gender. Geismar *et al.* (27) stated that the association between periodontal disease and CVD was dependent above all from age ($\geq$ 60 years old), but also from the diabetes mellitus and smoking habits, and not directly from both pathologies themselves. In contrast, a second study conducted on Irish participants showed a link between periodontal disease and CVD independent from its risk factors, namely diabetes mellitus (28). In our study there was an association between both pathologies and increasing age, as expected from the literature, but an association with the remaining risk factors was not clear, suggesting then an increase in the severity of periodontitis could be a risk factor that could aggravate the atherosclerotic condition.

The dental plaque index (bacteria) described in the literature as a risk factor for periodontal disease, showed in our study as an influencing factor for presence of periodontitis, but related more with slight and moderate forms of periodontitis, and without any influence over the remaining variables. This is in agreement with other studies in which the individual susceptibility of each patient was highlighted. This fact indicating that, although bacteria plaque is necessary at the beginning of periodontitis and during its progression, and in periodontal disease in general, the severity of the periodontitis will be dependent on systemic risks and patient behavior (29,30). Owing to the fact that there are only six smokers in the study, there is not enough power to detect a difference between groups.

In conclusion, the association found between periodontitis severity and carotid atherosclerosis, including subclinical, suggests that periodontal disease might be a risk factor for atherosclerotic disease.

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Conflict of interest and sources of funding

The authors declare no conflict of interests. The study was self-supported.

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