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CHRONIC PERIODONTITIS IS ASSOCIATED TO ERECTILE DYSFUNCTION. A CASE-CONTROL STUDY IN EUROPEAN POPULATION

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Abstract

Aim: To determine the association between chronic periodontitis and erectile dysfunction adjusting for biochemical markers and other co-morbidities.

Methods: A case-control study was conducted on 158 male patients. 80 cases with erectile dysfunction according to the International Index of Erectile Function, and 78 controls. Sociodemographic data were gathered and a periodontal examination was performed. Testosterone, lipid profile, C-reactive protein and glycaemic parameters were assessed. All variables were compared between groups and multivariate logistic regression analyses were performed.

Results: 74% of the cases were diagnosed with chronic periodontitis. Number of sites with pocket probing depth 4-6 mm (p=0.05) and number of sites with clinical attachment loss >3 mm (p<0.01) were higher in the cases. Triglycerides (p<0.01), C-reactive protein (p=0.02) and glycosylated haemoglobin (p=0.04) were also higher in the cases. Logistic regression showed that patients with chronic periodontitis were more likely to have erectile dysfunction (OR=2.17; 95%CI (1.06-4.43); p=0.03) independently of other confounders.

Conclusion: Patients with erectile dysfunction showed worse periodontal condition. Chronic periodontitis seems to play a key role as a risk factor in the pathogenesis of erectile dysfunction independently of other morbidities.

Clinical Relevance

Scientific rationale for study: Evidence on the relationship between chronic periodontitis and erectile dysfunction has not been analysed in European population accounting for biochemical markers.

Principal findings: Chronic periodontitis was a risk factor for erectile dysfunction.

Practical implications: Periodontitis should be considered as an important parameter by urologists. Periodontal treatment could be of help in the prevention and treatment of erectile dysfunction.

Introduction

There is increasing evidence that chronic periodontitis (CP) can cause endothelial dysfunction, the first step of vascular pathology, expressed by smaller values of flow-mediated dilation of the brachial artery. Periodontal bacteria or higher systemic levels of proinflammatory cytokines would cause a dysregulation in the endothelium (Moura et al. 2017, Punj et al. 2017). Atheroma plaque formation at the intima of medium and small vases would be the result of this dysregulation. Although this is a known mechanism, recent research has strengthened the relationship between periodontopathic anaerobic bacteria and atherosclerosis (Mahalakshmi et al. 2017). Based on this pathogenic mechanism, it has been accepted that CP is a risk factor for cardiovascular disease (CVD), and the extent and severity of CP has been positively associated with acute myocardial infarct size as measured by serum troponin I and myoglobin levels (Marfil-Alvarez et al. 2014).

Erection is a neurovascular phenomenon that culminates in an increase of arterial flow within the hypogastric-penile bed, followed by the activation of the veno-occlusive mechanism of the *corpora cavernosa* in a hormonal and psychological environment (Lue 2000). Erectile dysfunction (ED) is defined as the inability of the male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse (NIH Consensus Conference 1993). It presents a 24% prevalence in general male population (Montorsi et al. 2010). ED may be of organic causes (e.g., vascular, neurogenic, hormonal, anatomic, or drug-induced), psychological causes (depression, anxiety, stress), or a combination of both (Heidelbaugh 2010). Vlachopoulos et al. reported a 47% prevalence of ED in patients with CVD, higher than the expected 24% in general population. In addition, they showed that ED severity and time from diagnosis was related to the severity of the coronary artery diseases, measured by angiography (Vlachopoulos et al. 2013).

Regarding the available evidence about CP and ED, 15 articles have addressed this topic. All of them have been published in the last 8 years and have been conducted in Asian population, including 2 recent metanalyses (Wang et al. 2016, Liu et al. 2017). Although both metanalyses showed a positive association between CP and ED, they also reported a significant heterogeneity among the studies (I^2=97.8% and 98% respectively). Hence, they both conclude that current evidence of the issue has limited quality and they point out the need for further larger and epidemiological controlled studies of homogeneous populations to better estimate the true role of CP as risk factor and the effects of this interaction. The only published clinical trial was conducted on Turkish population and showed that periodontal treatment improved the severity of ED (Eltas et al. 2013). This relationship has been explained by an endothelial dysfunction that also affects penis vascularization (Sharma et al. 2011).

The objective of our observational study was to assess the association of CP and ED, through the comparison with associated clinical and biochemical variables.

Materials and methods

An observational case-control study was designed on patients from the Urology Service of the "San Cecilio" University Hospital (Granada, Spain) from January 2015 to June 2017. Cases were patients newly diagnosed with ED from the andrology unit, according to the International Index of Erectile Function (IIEF) (Rosen et al. 2002). Control patients were recruited in the same dates in the urology service that attended for urologic causes not related to ED, such as phimosis, vasectomy, lithotripsy, renal calculi, benign prostatic hyperplasia or family planning. All controls were selected and matched for age with cases (±3 years). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed for the preparation of this manuscript (von Elm et al. 2008).

Inclusion criteria were age between 18 and 70 years and presence of at least 11 teeth in mouth. Patients were excluded if they had received previous periodontal treatment, if they were in treatment with atenolol, hydrochlorothiazide or hypertensive without treatment (possible ED as side effect), if they had received antibiotic or anti-inflammatory therapy in the two months prior to the examination, if they presented any diagnosed psychiatric disorder, and if they presented neoplastic diseases, HIV or other severe systemic infection. The procedures in this study were in accordance to the Declaration of Helsinki, as revised in 2013. The study was approved by the Research Ethics Committee of the "San Cecilio" University Hospital (Ref. C-10.12). All participants signed written informed consent when recruited.

According to Sample Power 2.0 (SPSS Inc., Chicago, IL), 63 cases and 63 controls were necessary to detect a standardized difference of 0.5 according to Cohen's scale, in the quantitative study variables (biochemical, periodontal etc.), with a power of 80% and an alpha-error of 5%. With the sample size achieved in our study (80 cases and 78 controls) statistical power increased to 88%.

Sociodemographic data were gathered from each patient: age, alcohol consumption (grs. /day) according to Standard Drink measurement adjusted to Spanish population, tobacco consumption (cigs. /day), diabetes and CVD related pathology (hypertension, history of past myocardial infarction, stroke or *Angina pectoris*). IIEF is an internationally accepted questionnaire-based index. It gives a score between 1 and 30 and can evaluate, among other aspects, the erectile function. Diagnosis of ED is considered with a score of 25 or less in the IIEF (Rosen et al. 2002).

Periodontal examination was performed using a frontal medical examination headlight (Heine Optotechnik GmbH & Co. KG, Herrsching, Germany), SE plus® mouth mirror (Hahnenkratt E. GmbH, Königsbach-Stein, Germany) and PCPUNC15 periodontal probe (Hu-Friedy, Chicago, IL, USA). Pocket probing depth (PPD) was measured in millimeters in 6 sites per tooth (mesiovestibular, vestibular, distovestibular, mesiolingual, lingual and distolingual). Gingival recession was measured and Clinical Attachment (CA) loss was calculated in millimeters. Bleeding on Probing (BoP) was registered in percentage according to the Ainamo and Bay index (Ainamo and Bay 1975). Presence of supragingival plaque was determined by the binary index of Tonetti (Tonetti 2002). The number of present teeth was also recorded. The severity of periodontitis was evaluated using a modification of the Periodontal Inflammatory Severity Index, referred to hereafter as the PISIM (Marfil-Alvarez et al. 2014). The PISIM score is the sum of the product of the number of sites and the PPD at each site divided by the number of remaining teeth ($PISIM=\sum (d_i\, n_i)/t$), where "i" is the site, "d" is the PPD of the site in mm, "n" is the absolute frequency of the sites, and "t" is the number of remaining teeth. All clinical examinations were performed by the same researcher (A.M.), who was previously calibrated with F.M. Intraclass correlation coefficients were 0.79 for inter-examiner agreement for PPD, accepting +/- 1mm variability. The researcher was blinded during the whole process since the recruitment was performed by a different urologist researcher (M.A.). Periodontitis was diagnosed when four or more teeth showed one or more sites with active BoP, PPD ≥4 mm and CA loss ≥3 mm, based on the index proposed by Lopez et al (Lopez et al. 2002).

Serum samples were obtained from each subject and levels of testosterone (ng/dL), C-reactive protein (CRP) (mg/L), total cholesterol (mg/dL), triglycerides (mg/dL), LDL (mg/dL), HDL (mg/dL), glucose (mg/dL), glycosylated haemoglobin (HbA1c) (%) were measured.

Data analysis was performed with a specific statistical software (SPSS for Windows v.20 (IBM Inc., Chicago, IL, USA)). Sociodemographic, periodontal, urologic and biochemical variables were compared between both groups. Note that to compare quantitative variables Mann-Whitney test was used, after checking the non-normality distributions for almost all variables (results not shown) with Shapiro-Wilk test. Finally, in order to identify the factors associated with being an ED patient, a multivariate logistic regression model was constructed. Potential variables were those presented in Tables 1, 2 and 3, with a bivariate $p<0.20$, and excluding those with association (measured by rs>0.70 to avoid collinearity). A backward stepwise method based on statistical significance was used, with p>0.10 to exclude a variable. A $p\leq 0.05$ significance level was considered in all tests. Statistical tests used are described in table footnotes.

Results

A total of 158 men were included in the study: 80 cases with ED and 78 controls. Table 1 describes and compares sociodemographic variables between both groups. Only the presence of diabetes (p=0.04) and CVD (p=0.04), analysed as binary variables, were higher in the case group.

Table 2 shows the comparisons between the studied biochemical variables. Triglycerides, CRP and HbA1c levels were significantly higher in the case group (p<0.01, p=0.02 and p=0.04, respectively). Glycaemia was close to statistical significance (p=0.08). Biochemical variables were categorized according to standard classifications of the National Institutes of Health.

Table 3 describes the results of the comparison between periodontal clinical variables. Oral hygiene, BoP and present teeth number were similar among groups. Case group showed a higher number of sites with PPD 4-6 mm and higher number of sites with CA loss > 3 mm (p=0.05 and p<0.01, respectively). According to the periodontitis criteria by Lopez et al., there was a 74% of periodontitis in the case group, *versus* a 58% in the control group (p=0.05). Regarding Spearman correlation analysis between ED measured as quantitative variable (IIEF score) and periodontal variables, there was an inverse significant correlation between the number of sites with PPD 4-6 mm, the number of sites with CA loss >3 mm, PISIM, and periodontitis (Lopez) (all of them p<0.05, data not shown).

Table 4 shows the results of the multivariate logistic regression analysis. Results showed that CP is an independent risk factor of ED. Periodontitis patients are more likely to present ED (OR=2.17), after adjusting for other risk factors. This likelihood was greater than the one showed by CVD or the upper limit of triglycerides.

Discussion

Our results showed an association between CP and ED. This association was independent of other known comorbidities. Men with CP were 2.17 times more likely to present ED compared to periodontally healthy men.

The significance of the association between CP and other pathologies can be modified by the definition of periodontitis used (Manau et al. 2008). In this study, we adopted a strict definition of periodontitis, as proposed by Lopez et al., that has been previously used by our group (Mesa et al. 2013) and other authors (Gomes-Filho et al. 2007). This definition requires the presence of 4 or more teeth showing one or more sites with PPD ≥4 mm, CA loss ≥3 mm, and BoP at the same site (Lopez et al. 2002). In addition, 3 other periodontal variables (sites with PPD 4-6 mm,

PISIM and CA loss >3mm) were also higher in the case group. This variables also showed a significant inverse correlation with IIEF score, making the association more robust.

A total of 15 studies on this topic have been published in the recent years, all of them conducted in Asian population. Leaving aside the one published by Matsumoto et al., in which they evaluate periodontal condition through an e-mail questionnaire (Matsumoto et al. 2014), only 2 observational studies perform a proper assessment of the periodontal status (Sharma et al. 2011, Oguz et al. 2013). Their results are in agreement with the ones of our study, but we would like to make some remarks. Both studies are conducted in a very young sample (mean age of 35 years, range 30-40 years), where ED prevalence would be very low (Krane et al. 1989). Also, periodontitis cases in such a young population may be considered as aggressive periodontitis, with several characteristics that differ from CP. It should also be considered that both studies do not account for confounders, and Sharma et al. do not use a control group for comparison (Sharma et al. 2011).

To our knowledge, this is the first study in European population and the first that determines plasma biochemical markers, since some of them can be common indicators and risk factors for both pathologies.

CRP is an acute-phase protein that in secreted in plasma by the liver. It is a non-specific but extremely sensitive biomarker of systemic inflammation and infection. It is currently considered by the scientific literature as risk factor for CVD and subclinical vascular disease (Emerging Risk Factors Collaboration et al. 2010, Prugger et al. 2013). Both CP and ED have also been associated with higher plasma CRP levels (Tuter et al. 2007, Gomes-Filho et al. 2011, Solak et al. 2014). In our study, a 48% of the cases presented elevated levels of CRP (above 3 mg/L), compared to 31% of controls, indicating a high CVD risk. Higher CVD was also observed in the ED group (54% prevalence), and CVD patients were more likely to present ED (OR=1.89; p=0.09). Considering that 74% of ED patients were diagnosed of CP, periodontal infection could contribute to endothelial dysfunction of small vessels first, such as the ones of the penile vasculature. Larger vessels could be affected after, like the coronary arteries.

A recent study on 3990 men with a 15 year follow-up has reported that hypertriglyceridemia was present in a 15-21% of the subjects diagnosed with ED, with an impaired penile blood flow. Authors conclude that high triglycerides is a predictor of atherogenic ED (Corona et al. 2016). The role of moderately elevated triglycerides in the pathophysiology of vaso-occlusive events has also been described (Lamarre et al. 2013), and post-breakfast triglycerides was a determinant of blood viscosity (Minato et al. 2017). This could cause a slowdown of the blood flow and, hence, affect small vessels. In our study, ED group presented higher triglycerides

compared to controls, and a 19% had levels above 200 mg/dL, being an independent predictor of ED.

The most common cause of ED is vascular disease (Chiurlia et al. 2005). Although IIEF cannot distinguish between vasculogenic, hormonal or psychogenic ED, our results refer to ED of vascular origin. Our results exclude other possible causes and therefore diagnose ED of vascular origin by exclusion. Mean testosterone levels were normal (>350 ng/dL) and they were similar when comparing both study groups. None of the participants of the study received therapy for anxiety, stress or depression. The most accurate diagnosis of vasculogenic ED is obtained by Echo-colour Doppler (with an intracavernous injection of Alprostadil and contrast solution) or nocturnal penile tumescence monitoring. Both test were considered invasive and were not performed by ethical reasons.

CP and ED share a number of systemic risk factors and co-morbidities such as age, smoking, diabetes and CVD (Roth et al. 2003, Chew et al. 2009). Both age and smoking habit were similar between both groups. Diabetes was evaluated as binary variables, and trough glucose and HbA1c levels. 34% of ED patients had plasma glucose levels above 100 mg/dL (*versus* 24% of controls) and 16% had HbA1c levels above 6.5% (*versus* 8% of controls), diagnostic of diabetes according to the American Diabetes Association (International Expert Committee 2009). Keller et al. and Tsao et al. reported similar results in Taiwanese population but they conducted both studies using data from a national health database. They only use diabetes as a binary variable, without reporting data about glycaemia or HbA1c, and they do not analyse diabetes as an individual predictor of ED (Keller et al. 2012, Tsao et al. 2015). Although finding statistical significance in the bivariate analysis, after adjusting for confounders (see material and methods), diabetes does not play a role in the association between CP and ED. In addition, diabetes was not a predictor of ED. This finding may be explained by the fact that all diabetic patients were in treatment and that, even with higher mean levels of HbA1c in the case group, they were not high enough, probably due to the therapy. CP impairs glycaemic control and, since all cases of CP in this study were not treated, this could also explain that diabetic patients did not achieve normal HbA1c targets (Teeuw et al. 2010). Diabetes has been considered a direct cause of ED, explained by penile vessels microangiopathy. However, the available evidence reports higher HbA1c levels than the ones of our study (>8%) as predictors of ED (Rhoden et al. 2005).

Only one clinical trial to this date, conducted in Turkish population, has evaluated the effect of non-surgical periodontal therapy on ED. They reported a significant improvement in periodontal health and IIEF scores in the treatment group after 3 months of follow-up (Eltas et al. 2013). These results support the hypothesis that periodontal treatment would reduce systemic

inflammation (following the previously stated relationship between CP and systemic biomarkers of inflammation) and indirectly improve erectile function.

Among the limitations of this study, some of them are derived from the epidemiological design of this study, a case-control study. In this type of study, selection bias (in cases and/or controls) and/or confusion bias are always a possibility. Although it could be argued that controls should be healthy people from the general population, according to Rothman, when selecting controls the main target should be subjects representative of the one that may become cases of the study. This principle is fulfilled in our study. It should be also taken into consideration the difficulty or even impossibility that may have recruiting control patients outside of the context of a urology setting. Regarding the possible presence of confusion bias, multivariate analysis was performed adjusting for known risk factors, although this analysis is always limited only known factors. Another potential limitation is the use of a self-administered questionnaire (such as IIEF) for the diagnosis of ED. Although internationally validated, it could be difficult to understand and answer for patients of low socio-economic status. The IIEF is focused on the penetration ability and ignores other aspects of sexual life, and it does not distinguish between the different aetiologies of ED. To achieve a better understanding of the interaction between CP and vasculogenic ED, longitudinal studies that assess penile endothelial dysfunction with an objective technique are needed. Flow-mediated dilation assessment, considered the gold standard tool in vascular epidemiology (Punj et al. 2017), would be an option. Our results, within the limitations of an observational study, support the idea of including oral health as one aspect to be considered by urologists in the treatment of ED.

Conclusions

Our results showed an association between CP and ED. ED patients showed worse periodontal condition. CP seems to play a key role as a risk factor in the pathogenesis of ED, independently of other morbidities.

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Conflict of interest

The authors report no conflicts of interest related to this study.

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Table 1. Descriptive and bivariate comparisons of patient characteristics between study groups (n=158).

Variable	Controls (n=78)	Cases (n=80)	*p*-value
IIEF score, mean±sd	28±1	13±7	<0.01[b]
6-10 (severe), %	0	44	
11 to 16 (moderate), %	0	26	
17 to 25 (mild), %	0	30	
26 to 30 (No DE), %	100	0	
Age (yrs.), mean±sd	53±8	53±9	0.75[b]
23 to 39, %	6	8	
40 to 49, %	24	21	
50 to 59, %	49	51	
60 to 69, %	21	20	
Tobacco, %			
Never smoker	40	33	0.22[b]
Ex-smoker ≥11 yrs.	24	21	
Ex-smoker ≤10 yrs.	15	21	
1 to 14 cigs. /day	13	12	
≥15 cigs. /day	8	12	
Alcohol (grs. /day), mean±sd	10±15	9±10	0.68[b]
0, %	37	35	
>0 to <10, %	36	33	
10 to <20, %	10	17	
20 to 96, %	17	15	
Diabetes, %			
No	90	76	0.04[a]

Yes	10	24	
Cardiovascular disease, %			
No	64	46	0.04[a]
Yes	36	54	

All values apart from means are percentages expressed without decimals; a: Chi square test (with continuity correction); b: Mann-Whitney test for all other variables.

Table 2. Bivariate comparison of biochemical variables between study groups (n=158).

Variable	Controls (n=78)		Cases (n=80)		p-value[b]
	n[a]		n[a]		
Testosterone (ng/dL), mean±sd	61	384±140	70	359±107	0.69
<300 (low), %		23		26	
300 to 1000 (normal), %		77		74	
Total Cholesterol (mg/dL), mean±sd	73	196±40	79	200±46	0.82
<200 (normal), %		48		54	
200 to 239 (borderline high), %		40		25	
≥240 (high), %		12		20	
Triglycerides (mg/dL), mean±sd	73	128±87	78	151±75	<0.01
<150 (normal), %		78		60	
150 to 199 (borderline high), %		10		21	
≥200 (high), %		12		19	
HDL-cholesterol (mg/dL), mean±sd	71	52±12	78	50±10	0.41
<40 (low), %		14		15	
40 to 59 (normal), %		63		64	
≥60 (high), %		23		21	
LDL-cholesterol (mg/dL), mean±sd	71	120±35	77	123±40	0.92
<100 (optimal), %		28		30	
100 to 129 (near optimal), %		31		31	
130 to 159 (borderline high), %		30		25	
≥160 (high), %		11		14	
C-reactive protein (mg/L), mean±sd	65	3.24±4.69	73	4.47±5.23	0.02
<1 (low risk), %		23		18	

1 to 3 (medium risk), %		46		34	
>3 (high risk), %		31		48	
Glycemia (mg/dL), mean±sd	74	93±19	79	105±43	0.08
<72 (low), %		3		3	
72-100 (normal), %		73		63	
>100 (high), %		24		34	
HbA1c (%), mean±sd	65	5.60±0.69	69	5.86±0.91	0.04
<5.7 (normal), %		71		58	
5.7-6.4 (alto riesgo), %		21		26	
≥6.5 (diabetes), %		8		16	

a: The differences with total controls or cases correspond to missing data; b: Mann-Whitney test. All percentages expressed without decimals.

Table 3. Comparison of periodontal variables between study groups (n=158).

Variable	Controls (n=78)	Cases (n=80)	*p*-value
Tooth count, mean±sd	23±5	23±4	0.31[b]
Plaque index (%), mean±sd	67±31	69±29	0.52[b]
BoP (%), mean±sd	30±24	32±26	0.69[b]
Sites PPD 4-6 mm, mean±sd	13.9±17.9	19.0±20.9	0.05[b]
Sites PPD ≥7 mm, mean±sd	0.2±0.8	0.4±1.3	0.45[b]
PISIM, mean±sd	2.79±3.93	3.90±4.73	0.06[b]
Sites CA loss >3 mm., mean±sd	23±24	35±31	<0.01[b]
Periodontitis, %			0.05[a]
No	42	26	
Yes	58	74	

Percentages expressed without decimals; a: Chi square test (with continuity correction); b: Mann-Whitney test.

Table 4. Logistic regression model for the association of erectile dysfunction (n=158 patients: 78 controls + 80 cases)[a]

Variable	n	β±se	OR[b] (95% CI)	*p*-value
Diabetes				0.18
No (Reference)	131	0.00	1.00	
Yes	27	0.65±0.49	1.92 (0.74-5.00)	
Cardiovascular disease				0.09
No (Reference)	87	0.00	1.00	
Yes	71	0.61±0.36	1.83 (0.91-3.69)	
Triglycerides (mg/dL)				0.13
<150 (normal) (Reference)	104	0.00	1.00	-
150 to 199 (borderline high)	23	1.01±0.51	2.74 (1.00-7.47)	0.05
≥200 (high)	24	0.60±0.49	1.82 (0.69-4.81)	0.23
Missing	7	-0.60±0.88	0.55 (0.10-3.04)	0.49
Periodontitis				0.03
No (Reference)	54	0.00	1.00	
Yes	104	0.77±0.36	2.17 (1.06-4.43)	

a: See methods for a description of the strategy for building the model; b: OR=Odds ratio (e^{β}).

Manuscript Title: Periodontitis is associated to erectile dysfunction. A case-control study in European population **(CPE-01-18-7496).**

Reviewer(s)' Comments to Author:

Referee: 1

1. The last sentence of the result section in the abstract must be removed.
   Response: We remove that sentence following the reviewer's instructions.

2. Why the presence of eleven teeth was taken as a criterion in the method section.
   Response: The chosen number did not follow any specific motivation. Our objective was to enroll patients with a number of present teeth (a little bit more than a quadrant) that allowed to record data that gave enough consistency to the periodontal status of the patient. We think that the quality of the gathered data would have been lower if the patients only had few teeth present in mouth. This, along with exploring 6 sites per tooth, was performed to obtain data of high quality for the analysis.

3. No power analysis. It should be added.
   Response: Following the reviewer's instructions, we have added the following paragraph in the methods section about the power analysis:

   "According to Sample Power 2.0 (SPSS Inc., Chicago, IL), 63 cases and 63 controls were necessary to detect a standardized difference of 0.5 according to Cohen's scale (Cohen J. Statistical power analysis for the behavioral sciences. Hillside, New Jersey: Lawrence Erlbaum Associates, 1988), in the quantitative study variables (biochemical, periodontal etc.), with a power of 80% and an alpha-error of 5%. With the sample size achieved in our study (80 cases and 78 controls) statistical power increased to 88%."

   The reference has not been cited because article has currently 40 references (maximum stated in the guidelines). If the reviewer and the editor consider it necessary and authorize it, we can add this citation to the references.

4. The symbol ")" in Table 2 should be removed.
   Response: Symbol has been removed since it was a mistake.

5. What is the purpose of distinguishing between subgroups of data in Table 2?

   Response: Table 2 includes the means, but also the percentage distributions of each variable. The cut-off points used for this purpose were based on clinical significance. We have presented data in this way in order to improve the description and the interpretation of our findings.

Referee: 2

1. The major concern of this research is diagnosis of ED. As this research is based on the concept of Vascular ED, patients were not diagnosed particularly on this basis.

   Response: As we state in discussion (page 8): "IIEF cannot distinguish between vasculogenic, hormonal or psychogenic ED". Our results can refer to an ED of vascular origin by exclusion of other causes. Mean testosterone levels were normal and there were no patients with diagnosed psychiatric or neurologic disorders. The real confirmation of vascular ED, as we also state in discussion, requires the use of invasive diagnostic tests, which are not ethically justified. We add a comment in this section (page 8) for more clarity:

   "Our results exclude other possible causes and therefore diagnose ED of vascular origin by exclusion"

2. What were the criteria for diagnosing vascular ED? Clinically it cannot be categorized.

   Response: As stated in the previous question, the assumption of vascular ED diagnosis was based on the exclusion of other possible causes. The only possible confirmation was by invasive tests that were not ethically justified, since ED therapy is the same, independently of the cause.

3. Exclusion/inclusion criteria do not have consideration for antipsychotic and/or related drugs.

   Response: We confirm that none of the participants of the study had diagnosis of mental disease and, as we state in discussion: "None of the participants of the study received therapy for anxiety, stress or depression". This was done in order to avoid ED cases of psychogenic origin. We agree with the referee that this should be included as

exclusion criteria. We included a sentence regarding this issue in material and methods (page 4): *"...if they presented any diagnosed psychiatric disorder..."*

4. Which all severe inflammatory diseases excluded from this study. Other diseases like epilepsy, CKD, lung diseases or any other diseases were not excluded.
   Response: We include as exclusion criteria diagnosis of infectious diseases that could mask chronic periodontitis. The study was performed in a urologic primary care setting, in cases with first diagnosis of ED, being also ED the main reason of the visit. Patients with lung, kidney, and liver disorders were not included.

5. Controls must have been systemically healthy. Though biochemical markers and other comorbidities were adjusted, but many confounders were not taken into consideration.
   Response: We have included an answer about this issue in the limitations of the study (please see query 7 below).

6. Kindly elaborate on statistically significant association of CP and ED in relation to Diabetes and CVD association in Table 1.
   Response: Table 1 does not show data related to periodontal clinical variables. It only describes the unadjusted associations of several risk factors for ED. We believe that the question of the referee is about the possible role of diabetes and CVD as predictors in the relationship between CP and ED. This issue is addressed in discussion section (page 8), where the results of the multivariate analysis (table 4) are discussed. Here it is highlighted that both diabetes and CVD do not play any role in the association between CP and ED, since the significance is lost in the multivariate analysis.

7. Kindly include all limitations of this study.
   Response: In addition to the limitations already included in the manuscript (page 9), we add more data to this paragraph regarding this issue:

   "A potential limitation derived from the epidemiological design of this study, a case-control study. In this type of study, selection bias (in cases and/or controls) and/or confusion bias are always a possibility. Although it could be argued that controls should be healthy people from the general population, according to Rothman, when selecting controls the main target should be subjects representative of the one that may become cases of the study (Rothman KJ. Modern Epidemiology. Boston: Little, Brown, 1986). This principle is fulfilled in our study. It should be also taken into consideration the difficulty or even impossibility that may have recruiting control patients outside of the context of a urology setting. Regarding the

possible presence of confusion bias, multivariate analysis was performed adjusting for known risk factors, although this analysis is always limited only known factors."

The reference from Rothman has not been cited in the text because article has currently 40 references (maximum stated in the author guidelines). If the reviewer and the editor consider it necessary and authorize it, we can add this citation to the references.

CHRONIC PERIODONTITIS IS ASSOCIATED TO ERECTILE DYSFUNCTION. A CASE-CONTROL STUDY IN EUROPEAN POPULATION

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Abstract

Aim: To determine the association between chronic periodontitis and erectile dysfunction adjusting for biochemical markers and other co-morbidities.

Methods: A case-control study was conducted on 158 male patients. 80 cases with erectile dysfunction according to the International Index of Erectile Function, and 78 controls. Sociodemographic data were gathered and a periodontal examination was performed. Testosterone, lipid profile, C-reactive protein and glycaemic parameters were assessed. All variables were compared between groups and multivariate logistic regression analyses were performed.

Results: 74% of the cases were diagnosed with chronic periodontitis. Number of sites with pocket probing depth 4-6 mm (p=0.05) and number of sites with clinical attachment loss >3 mm (p<0.01) were higher in the cases. Triglycerides (p<0.01), C-reactive protein (p=0.02) and glycosylated haemoglobin (p=0.04) were also higher in the cases. Logistic regression showed that patients with chronic periodontitis were more likely to have erectile dysfunction (OR=2.17; 95%CI (1.06-4.43); p=0.03) independently of other confounders.

Conclusion: Patients with erectile dysfunction showed worse periodontal condition. Chronic periodontitis seems to play a key role as a risk factor in the pathogenesis of erectile dysfunction independently of other morbidities.

Clinical Relevance

Scientific rationale for study: Evidence on the relationship between chronic periodontitis and erectile dysfunction has not been analysed in European population accounting for biochemical markers.

Principal findings: Chronic periodontitis was a risk factor for erectile dysfunction.

Practical implications: Periodontitis should be considered as an important parameter by urologists. Periodontal treatment could be of help in the prevention and treatment of erectile dysfunction.

Introduction

There is increasing evidence that chronic periodontitis (CP) can cause endothelial dysfunction, the first step of vascular pathology, expressed by smaller values of flow-mediated dilation of the brachial artery. Periodontal bacteria or higher systemic levels of proinflammatory cytokines would cause a dysregulation in the endothelium (Moura et al. 2017, Punj et al. 2017). Atheroma plaque formation at the intima of medium and small vases would be the result of this dysregulation. Although this is a known mechanism, recent research has strengthened the relationship between periodontopathic anaerobic bacteria and atherosclerosis (Mahalakshmi et al. 2017). Based on this pathogenic mechanism, it has been accepted that CP is a risk factor for cardiovascular disease (CVD), and the extent and severity of CP has been positively associated with acute myocardial infarct size as measured by serum troponin I and myoglobin levels (Marfil-Alvarez et al. 2014).

Erection is a neurovascular phenomenon that culminates in an increase of arterial flow within the hypogastric-penile bed, followed by the activation of the veno-occlusive mechanism of the *corpora cavernosa* in a hormonal and psychological environment (Lue 2000). Erectile dysfunction (ED) is defined as the inability of the male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse (NIH Consensus Conference 1993). It presents a 24% prevalence in general male population (Montorsi et al. 2010). ED may be of organic causes (e.g., vascular, neurogenic, hormonal, anatomic, or drug-induced), psychological causes (depression, anxiety, stress), or a combination of both (Heidelbaugh 2010). Vlachopoulos et al. reported a 47% prevalence of ED in patients with CVD, higher than the expected 24% in general population. In addition, they showed that ED severity and time from diagnosis was related to the severity of the coronary artery diseases, measured by angiography (Vlachopoulos et al. 2013).

Regarding the available evidence about CP and ED, 15 articles have addressed this topic. All of them have been published in the last 8 years and have been conducted in Asian population, including 2 recent metanalyses (Wang et al. 2016, Liu et al. 2017). Although both metanalyses showed a positive association between CP and ED, they also reported a significant heterogeneity among the studies (I^2=97.8% and 98% respectively). Hence, they both conclude that current evidence of the issue has limited quality and they point out the need for further larger and epidemiological controlled studies of homogeneous populations to better estimate the true role of CP as risk factor and the effects of this interaction. The only published clinical trial was conducted on Turkish population and showed that periodontal treatment improved the severity of ED (Eltas et al. 2013). This relationship has been explained by an endothelial dysfunction that also affects penis vascularization (Sharma et al. 2011).

The objective of our observational study was to assess the association of CP and ED, through the comparison with associated clinical and biochemical variables.

Materials and methods

An observational case-control study was designed on patients from the Urology Service of the "San Cecilio" University Hospital (Granada, Spain) from January 2015 to June 2017. Cases were patients newly diagnosed with ED from the andrology unit, according to the International Index of Erectile Function (IIEF) (Rosen et al. 2002). Control patients were recruited in the same dates in the urology service that attended for urologic causes not related to ED, such as phimosis, vasectomy, lithotripsy, renal calculi, benign prostatic hyperplasia or family planning. All controls were selected and matched for age with cases (±3 years). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed for the preparation of this manuscript (von Elm et al. 2008).

Inclusion criteria were age between 18 and 70 years and presence of at least 11 teeth in mouth. Patients were excluded if they had received previous periodontal treatment, if they were in treatment with atenolol, hydrochlorothiazide or hypertensive without treatment (possible ED as side effect), if they had received antibiotic or anti-inflammatory therapy in the two months prior to the examination, if they presented any diagnosed psychiatric disorder, and if they presented neoplastic diseases, HIV or other severe systemic infection. The procedures in this study were in accordance to the Declaration of Helsinki, as revised in 2013. The study was approved by the Research Ethics Committee of the "San Cecilio" University Hospital (Ref. C-10.12). All participants signed written informed consent when recruited.

According to Sample Power 2.0 (SPSS Inc., Chicago, IL), 63 cases and 63 controls were necessary to detect a standardized difference of 0.5 according to Cohen's scale, in the quantitative study variables (biochemical, periodontal etc.), with a power of 80% and an alpha-error of 5%. With the sample size achieved in our study (80 cases and 78 controls) statistical power increased to 88%.

Sociodemographic data were gathered from each patient: age, alcohol consumption (grs. /day) according to Standard Drink measurement adjusted to Spanish population, tobacco consumption (cigs. /day), diabetes and CVD related pathology (hypertension, history of past myocardial infarction, stroke or *Angina pectoris*). IIEF is an internationally accepted questionnaire-based index. It gives a score between 1 and 30 and can evaluate, among other aspects, the erectile function. Diagnosis of ED is considered with a score of 25 or less in the IIEF (Rosen et al. 2002).

Periodontal examination was performed using a frontal medical examination headlight (Heine Optotechnik GmbH & Co. KG, Herrsching, Germany), SE plus® mouth mirror (Hahnenkratt E. GmbH, Königsbach-Stein, Germany) and PCPUNC15 periodontal probe (Hu-Friedy, Chicago, IL, USA). Pocket probing depth (PPD) was measured in millimeters in 6 sites per tooth (mesiovestibular, vestibular, distovestibular, mesiolingual, lingual and distolingual). Gingival recession was measured and Clinical Attachment (CA) loss was calculated in millimeters. Bleeding on Probing (BoP) was registered in percentage according to the Ainamo and Bay index (Ainamo and Bay 1975). Presence of supragingival plaque was determined by the binary index of Tonetti (Tonetti 2002). The number of present teeth was also recorded. The severity of periodontitis was evaluated using a modification of the Periodontal Inflammatory Severity Index, referred to hereafter as the PISIM (Marfil-Alvarez et al. 2014). The PISIM score is the sum of the product of the number of sites and the PPD at each site divided by the number of remaining teeth (PISIM=$\sum (d_i\, n_i)/t$), where "i" is the site, "d" is the PPD of the site in mm, "n" is the absolute frequency of the sites, and "t" is the number of remaining teeth. All clinical examinations were performed by the same researcher (A.M.), who was previously calibrated with F.M. Intraclass correlation coefficients were 0.79 for inter-examiner agreement for PPD, accepting +/- 1mm variability. The researcher was blinded during the whole process since the recruitment was performed by a different urologist researcher (M.A.). Periodontitis was diagnosed when four or more teeth showed one or more sites with active BoP, PPD ≥4 mm and CA loss ≥3 mm, based on the index proposed by Lopez et al (Lopez et al. 2002).

Serum samples were obtained from each subject and levels of testosterone (ng/dL), C-reactive protein (CRP) (mg/L), total cholesterol (mg/dL), triglycerides (mg/dL), LDL (mg/dL), HDL (mg/dL), glucose (mg/dL), glycosylated haemoglobin (HbA1c) (%) were measured.

Data analysis was performed with a specific statistical software (SPSS for Windows v.20 (IBM Inc., Chicago, IL, USA)). Sociodemographic, periodontal, urologic and biochemical variables were compared between both groups. Note that to compare quantitative variables Mann-Whitney test was used, after checking the non-normality distributions for almost all variables (results not shown) with Shapiro-Wilk test. Finally, in order to identify the factors associated with being an ED patient, a multivariate logistic regression model was constructed. Potential variables were those presented in Tables 1, 2 and 3, with a bivariate $p<0.20$, and excluding those with association (measured by rs>0.70 to avoid collinearity). A backward stepwise method based on statistical significance was used, with p>0.10 to exclude a variable. A $p\leq 0.05$ significance level was considered in all tests. Statistical tests used are described in table footnotes.

Results

A total of 158 men were included in the study: 80 cases with ED and 78 controls. Table 1 describes and compares sociodemographic variables between both groups. Only the presence of diabetes (p=0.04) and CVD (p=0.04), analysed as binary variables, were higher in the case group.

Table 2 shows the comparisons between the studied biochemical variables. Triglycerides, CRP and HbA1c levels were significantly higher in the case group (p<0.01, p=0.02 and p=0.04, respectively). Glycaemia was close to statistical significance (p=0.08). Biochemical variables were categorized according to standard classifications of the National Institutes of Health.

Table 3 describes the results of the comparison between periodontal clinical variables. Oral hygiene, BoP and present teeth number were similar among groups. Case group showed a higher number of sites with PPD 4-6 mm and higher number of sites with CA loss > 3 mm (p=0.05 and p<0.01, respectively). According to the periodontitis criteria by Lopez et al., there was a 74% of periodontitis in the case group, *versus* a 58% in the control group (p=0.05). Regarding Spearman correlation analysis between ED measured as quantitative variable (IIEF score) and periodontal variables, there was an inverse significant correlation between the number of sites with PPD 4-6 mm, the number of sites with CA loss >3 mm, PISIM, and periodontitis (Lopez) (all of them p<0.05, data not shown).

Table 4 shows the results of the multivariate logistic regression analysis. Results showed that CP is an independent risk factor of ED. Periodontitis patients are more likely to present ED (OR=2.17), after adjusting for other risk factors. This likelihood was greater than the one showed by CVD or the upper limit of triglycerides.

Discussion

Our results showed an association between CP and ED. This association was independent of other known comorbidities. Men with CP were 2.17 times more likely to present ED compared to periodontally healthy men.

The significance of the association between CP and other pathologies can be modified by the definition of periodontitis used (Manau et al. 2008). In this study, we adopted a strict definition of periodontitis, as proposed by Lopez et al., that has been previously used by our group (Mesa et al. 2013) and other authors (Gomes-Filho et al. 2007). This definition requires the presence of 4 or more teeth showing one or more sites with PPD ≥4 mm, CA loss ≥3 mm, and BoP at the same site (Lopez et al. 2002). In addition, 3 other periodontal variables (sites with PPD 4-6 mm,

PISIM and CA loss >3mm) were also higher in the case group. This variables also showed a significant inverse correlation with IIEF score, making the association more robust.

A total of 15 studies on this topic have been published in the recent years, all of them conducted in Asian population. Leaving aside the one published by Matsumoto et al., in which they evaluate periodontal condition through an e-mail questionnaire (Matsumoto et al. 2014), only 2 observational studies perform a proper assessment of the periodontal status (Sharma et al. 2011, Oguz et al. 2013). Their results are in agreement with the ones of our study, but we would like to make some remarks. Both studies are conducted in a very young sample (mean age of 35 years, range 30-40 years), where ED prevalence would be very low (Krane et al. 1989). Also, periodontitis cases in such a young population may be considered as aggressive periodontitis, with several characteristics that differ from CP. It should also be considered that both studies do not account for confounders, and Sharma et al. do not use a control group for comparison (Sharma et al. 2011).

To our knowledge, this is the first study in European population and the first that determines plasma biochemical markers, since some of them can be common indicators and risk factors for both pathologies.

CRP is an acute-phase protein that in secreted in plasma by the liver. It is a non-specific but extremely sensitive biomarker of systemic inflammation and infection. It is currently considered by the scientific literature as risk factor for CVD and subclinical vascular disease (Emerging Risk Factors Collaboration et al. 2010, Prugger et al. 2013). Both CP and ED have also been associated with higher plasma CRP levels (Tuter et al. 2007, Gomes-Filho et al. 2011, Solak et al. 2014). In our study, a 48% of the cases presented elevated levels of CRP (above 3 mg/L), compared to 31% of controls, indicating a high CVD risk. Higher CVD was also observed in the ED group (54% prevalence), and CVD patients were more likely to present ED (OR=1.89; p=0.09). Considering that 74% of ED patients were diagnosed of CP, periodontal infection could contribute to endothelial dysfunction of small vessels first, such as the ones of the penile vasculature. Larger vessels could be affected after, like the coronary arteries.

A recent study on 3990 men with a 15 year follow-up has reported that hypertriglyceridemia was present in a 15-21% of the subjects diagnosed with ED, with an impaired penile blood flow. Authors conclude that high triglycerides is a predictor of atherogenic ED (Corona et al. 2016). The role of moderately elevated triglycerides in the pathophysiology of vaso-occlusive events has also been described (Lamarre et al. 2013), and post-breakfast triglycerides was a determinant of blood viscosity (Minato et al. 2017). This could cause a slowdown of the blood flow and, hence, affect small vessels. In our study, ED group presented higher triglycerides

compared to controls, and a 19% had levels above 200 mg/dL, being an independent predictor of ED.

The most common cause of ED is vascular disease (Chiurlia et al. 2005). Although IIEF cannot distinguish between vasculogenic, hormonal or psychogenic ED, our results refer to ED of vascular origin. Our results exclude other possible causes and therefore diagnose ED of vascular origin by exclusion. Mean testosterone levels were normal (>350 ng/dL) and they were similar when comparing both study groups. None of the participants of the study received therapy for anxiety, stress or depression. The most accurate diagnosis of vasculogenic ED is obtained by Echo-colour Doppler (with an intracavernous injection of Alprostadil and contrast solution) or nocturnal penile tumescence monitoring. Both test were considered invasive and were not performed by ethical reasons.

CP and ED share a number of systemic risk factors and co-morbidities such as age, smoking, diabetes and CVD (Roth et al. 2003, Chew et al. 2009). Both age and smoking habit were similar between both groups. Diabetes was evaluated as binary variables, and trough glucose and HbA1c levels. 34% of ED patients had plasma glucose levels above 100 mg/dL (*versus* 24% of controls) and 16% had HbA1c levels above 6.5% (*versus* 8% of controls), diagnostic of diabetes according to the American Diabetes Association (International Expert Committee 2009). Keller et al. and Tsao et al. reported similar results in Taiwanese population but they conducted both studies using data from a national health database. They only use diabetes as a binary variable, without reporting data about glycaemia or HbA1c, and they do not analyse diabetes as an individual predictor of ED (Keller et al. 2012, Tsao et al. 2015). Although finding statistical significance in the bivariate analysis, after adjusting for confounders (see material and methods), diabetes does not play a role in the association between CP and ED. In addition, diabetes was not a predictor of ED. This finding may be explained by the fact that all diabetic patients were in treatment and that, even with higher mean levels of HbA1c in the case group, they were not high enough, probably due to the therapy. CP impairs glycaemic control and, since all cases of CP in this study were not treated, this could also explain that diabetic patients did not achieve normal HbA1c targets (Teeuw et al. 2010). Diabetes has been considered a direct cause of ED, explained by penile vessels microangiopathy. However, the available evidence reports higher HbA1c levels than the ones of our study (>8%) as predictors of ED (Rhoden et al. 2005).

Only one clinical trial to this date, conducted in Turkish population, has evaluated the effect of non-surgical periodontal therapy on ED. They reported a significant improvement in periodontal health and IIEF scores in the treatment group after 3 months of follow-up (Eltas et al. 2013). These results support the hypothesis that periodontal treatment would reduce systemic

inflammation (following the previously stated relationship between CP and systemic biomarkers of inflammation) and indirectly improve erectile function.

Among the limitations of this study, some of them are derived from the epidemiological design of this study, a case-control study. In this type of study, selection bias (in cases and/or controls) and/or confusion bias are always a possibility. Although it could be argued that controls should be healthy people from the general population, according to Rothman, when selecting controls the main target should be subjects representative of the one that may become cases of the study. This principle is fulfilled in our study. It should be also taken into consideration the difficulty or even impossibility that may have recruiting control patients outside of the context of a urology setting. Regarding the possible presence of confusion bias, multivariate analysis was performed adjusting for known risk factors, although this analysis is always limited only known factors. Another potential limitation is the use of a self-administered questionnaire (such as IIEF) for the diagnosis of ED. Although internationally validated, it could be difficult to understand and answer for patients of low socio-economic status. The IIEF is focused on the penetration ability and ignores other aspects of sexual life, and it does not distinguish between the different aetiologies of ED. To achieve a better understanding of the interaction between CP and vasculogenic ED, longitudinal studies that assess penile endothelial dysfunction with an objective technique are needed. Flow-mediated dilation assessment, considered the gold standard tool in vascular epidemiology (Punj et al. 2017), would be an option. Our results, within the limitations of an observational study, support the idea of including oral health as one aspect to be considered by urologists in the treatment of ED.

Conclusions

Our results showed an association between CP and ED. ED patients showed worse periodontal condition. CP seems to play a key role as a risk factor in the pathogenesis of ED, independently of other morbidities.

Acknowledgments

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Conflict of interest

The authors report no conflicts of interest related to this study.

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Servicio Andaluz de Salud
CONSEJERÍA DE SALUD

DON JUAN MORALES ARCAS, EN CALIDAD DE SECRETARIO DEL COMITÉ DE ÉTICA DE LA INVESTIGACIÓN BIOMÉDICA DE LA PROVINCIA DE GRANADA,

C E R T I F I C A

Que este Comité ha evaluado favorablemente, en su reunión celebrada el día 22 de diciembre de 2014, el proyecto, presentado por el Dr. Miguel Arrabal Martín, F. E. A. de Urología del Complejo Hospitalario Universitario de Granada (San Cecilio) y el Dr. Francisco Luis Mesa Aguado. Profesor Titular Departamento de Estomatología Universidad de Granada, titulado: "Relación entre periodontitis crónica y disfunción eréctil en población española". Código: C-10 diciembre.

Y considera que:

Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.

La capacidad del investigador y los medios disponibles son apropiados para llevar a cabo el mencionado estudio.

Es adecuado el procedimiento para obtener el consentimiento informado.

Y que este Comité acepta que dicho proyecto sea realizado en dichos Centros.

Lo que firmo en Granada, a veintidós de diciembre de dos mil catorce.

N.º72

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STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	X
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	X
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	X
Objectives	3	State specific objectives, including any prespecified hypotheses	X
Methods			
Study design	4	Present key elements of study design early in the paper	X
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	X
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	X
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	X
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	X
Bias	9	Describe any efforts to address potential sources of bias	X
Study size	10	Explain how the study size was arrived at	X
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	X
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	X
		(*b*) Describe any methods used to examine subgroups and interactions	X
		(*c*) Explain how missing data were addressed	X
		(*d*) If applicable, explain how matching of cases and controls was addressed	
		(*e*) Describe any sensitivity analyses	X
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	X
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	X
		(b) Indicate number of participants with missing data for each variable of interest	X
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	X
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates	X

		and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	X
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	X
Discussion			
Key results	18	Summarise key results with reference to study objectives	X
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	X
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	X
Generalisability	21	Discuss the generalisability (external validity) of the study results	X
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.