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Vitamin C in plasma and leucocytes in relation to periodontitis

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Abstract

Aim: To test the hypothesis that vitamin C concentrations in plasma, polymorphonuclear neutrophilic leucocytes (PMNs) and peripheral blood mononuclear cells (PBMCs) are lower in periodontitis patients compared with healthy controls.

Methods: Twenty-one untreated periodontal patients and 21 healthy controls matched for age, gender, race and smoking habits were selected. Dietary vitamin C intake was assessed by a self-administered dietary record. Fasting blood samples were obtained and analysed for vitamin C concentrations in plasma, PMNs and PBMCs by means of high-pressure liquid chromatography (HPLC).

Results: Plasma vitamin C was lower in periodontitis patients compared with controls (8.3 and 11.3 mg/l, respectively, $p = 0.03$). Only in the control group a positive correlation was present between vitamin C intake and plasma values. No differences could be assessed between patients and controls regarding vitamin C dietary intake and levels in PMNs and PBMCs. In the patient group, pocket depth appeared to be negatively associated with the vitamin C concentration in PMNs.

Conclusion: Although the relationship between low plasma vitamin C levels and periodontitis is clear, the disease cannot be explained by insufficient vitamin C storage capacity of leucocytes; the question remains through which mechanism low plasma vitamin C levels are related to periodontitis.

Key words: leucocytes; periodontitis; plasma; vitamin C

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For many years vitamin C (ascorbic acid) has been recognized to play a role in periodontal health which is supported by an inverse association between serum/plasma vitamin C concentrations and periodontitis (Amarasena et al. 2005, Panjamurthy et al. 2005, Staudte et al. 2005, Amaliya et al. 2007, Chapple et al. 2007). During the last decades there is growing evidence for the importance of vitamin C as antioxidant against reactive oxygen species (ROS) and in leucocyte function in healthy as well as periodontally diseased subjects (Chapple & Matthews 2007). Both ROS and immune cells are implicated in the pathophysiology of periodontitis (Kornman et al. 1997). It is known that leucocytes, in particular polymorphonuclear neutrophilic leucocytes (PMNs) and mononuclear cells (MNs), have a marked ability to concentrate ascorbic acid (Evans et al. 1982). The storage of vitamin C has been attributed to increased needs of these cells for the vitamin and suggests that it plays a significant role in normal cell function

Conflict of interest and source of funding statement

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(Boxer et al. 1979, Wolf 1993). Vitamin C aids in the bactericidal activity of PMNs and monocytes/macrophages, providing enhanced synthesis of nitric oxide (Sharma et al. 2003, 2004). It increases the chemotactic responsiveness of peripheral blood leucocytes and promotes the assembly of cellular microtubules involved in providing a structural framework for the cell (Boxer et al. 1979). In MNs, vitamin C has been suggested to increase glutathione (Lenton et al. 2003), to decrease oxidative DNA damage and to have a strong protective effect on the cells ex vivo (Panayiotidis & Collins 1997). However, most long-term intervention studies with vitamin C supplements failed to show an effect on various biomarkers of oxidative DNA damage (Welch et al. 1999).

Decreased leucocyte vitamin C concentrations have been shown in asthmatic patients (Shidfar et al. 2005) and in subjects with type 2 diabetes (Cunningham et al. 1991, Akkuş et al. 1996). It was suggested that intracellular vitamin C depletion may contribute to the chronic degenerative complications of the disease. At present, no studies are available on the intracellular storage of vitamin C in leucocytes of periodontitis patients. Provided vitamin C plays a protective role for the development and/or severity of periodontal disease as suggested in the literature, it may be hypothesized that its concentration in plasma, PMNs and peripheral blood mononuclear cells (PBMCs) is lower in periodontitis patients compared with healthy controls. Thus, the aim of the present investigation was to study the relationship between periodontitis, vitamin C intake and vitamin C as assessed in plasma, PMNs and PBMCs of untreated chronic periodontitis patients and healthy controls matched for age, gender, race and smoking habits.

Material and Methods

Study population

Twenty-one patients from the Academic Centre for Dentistry Amsterdam (ACTA) were selected on the basis of age (≥ 21 years), presence of radiographic bone loss >1/3 of the root length at ≥ 1 tooth per quadrant and ≥ 20 teeth. An equal number of controls matched for age, gender, race and smoking status were recruited if they presented with ≥ 20 teeth and a distance between the cemento-enamel junction and the inter-dental alveolar bone crest of ≤ 3 mm on dental radiographs not older than 12 months. Radiographic evaluation of bone levels was performed using a light box and a Shei ruler, scoring bone loss in categories of 10% increments relative to the total root length (Teeuw et al. 2009).

Exclusion criteria for patients and controls were: (1) presence of any systemic disease or chronic medical condition, apart from periodontitis, (2) recent history or presence of any acute or chronic infection, (3) tooth extractions or trauma in the last 2 weeks, (4) systemic antibiotic treatment within the last 6 months, (5) use of medication including NSAID's during the last 4 weeks, and (6) pregnancy.

All included subjects were informed both verbally and written about the purpose of the study and signed an informed consent to participate. The Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam approved the study.

Interview, dietary and clinical data

Demographic and medical histories were taken both by written questionnaires and a personal interview. Ethnicity was recorded and scored as Caucasian or non-Caucasian. For assessment of the education level, the reported highest level of education was used and classified into two categories i.e. less than high school education and high school education or higher. Tobacco smokers were defined as individuals who smoked ≥ 1 cigarette, pipe or cigar per day. The smoking habit was quantified by calculating pack years (number of packs per day multiplied by the number of years a person has smoked). Non-smokers were individuals who never smoked and those who had stopped smoking at least 6 months before entering the study. From height and weight data the body mass index (BMI) was calculated.

Nutrition was assessed by a self-administered dietary record. All participants were asked to fill in what they consumed 3 days prior to the examination and to answer questions about their dietary habits as well as the use of food supplements. The reported number of servings was obtained. Using the Netherlands Food Composition Tables (Nederlandse Voedingsmiddelentabel 2006) the different food and beverage items consumed were categorized as high, fair, low or no vitamin C when they provided >60 mg, 31–60 mg, 2–30 mg and <2 mg vitamin C/100 g respectively (a modification of the classification of Taylor et al. 2000). The total amount of vitamin C intake was calculated as the number of servings containing 2–30 mg/100 mg vitamin C plus two times the number of servings containing ≥ 31 mg/100 mg vitamin C (including supplements) during the previous 3 days. Clinical evaluation included assessment of plaque (present or absent), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL) recorded at six sites per tooth. From each patient a set of full-mouth dental radiographs was available. Teeth with $\geq 30\%$ and $\geq 50\%$ bone loss were identified radiographically to assess the severity of periodontal destruction.

Blood collection, cell isolation and analysis

Overnight fasting blood samples were obtained by venipuncture in the antecubital fossa. The blood was collected into 4 ml vacuum tubes (BD Vacutainer System, Plymouth, UK) containing ethylenediaminetetraacetic acid (EDTA) and used for automated leucocyte counting and differentiation in a hospital-based diagnostic clinical laboratory. In addition, venous blood was sampled in 9 ml vacuum tubes (Vacuette; Greiner Bio-One GmbH, Kremsmünster, Austria) containing lithium heparin as anticoagulant for vitamin C concentration measurements in plasma, PBMCs and PMNs.

Plasma was obtained by centrifugation with an Eppendorf centrifuge type 5804R (Eppendorf AG, Hamburg, Germany) at 2000 *g* at 4°C for 10 min. The PBMCs were isolated by a differential centrifugation method using a commercial medium

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(Axis-Shield PoC AS, Lymphoprep™, Oslo, Norway). Briefly, whole blood was diluted 1:1 with phosphate buffered saline (PBS) plus 1% citrate before being carefully layered over Lymphoprep™. After centrifugation at 800 *g* for 30 min. at room temperature a distinct cloudy band containing PBMCs and a red pellet containing erythrocytes and granulocytes were formed. The PBMCs were harvested from the cloudy band and transferred into a fresh tube, washed with PBS/citrate solution and centrifuged at 400 *g* for 10 min. to pellet the cells. The supernatant was aspirated and the cell pellet re-suspended in PBS. The PMNs were isolated from the red pellet. To remove the erythrocytes, the pellet was re-suspended in NH_4Cl lysis buffer and incubated on ice until the solution became translucent, indicating that the erythrocytes were lysed. The remaining cells (approximately 90% viable PMNs) were subsequently washed once in the same lysis buffer and re-suspended in PBS.

After purification all isolated specimens were subdivided into several aliquots in light protected reaction vials. According to the instruction manual for the high-pressure liquid chromatography (HPLC) analysis of vitamin C in plasma/serum, precipitation mix (90 μl) was added to all samples (90 μl) to minimize degradation of vitamin C and liberate intracellular vitamin C stores by cell lysis. Samples were mixed for 10 s and frozen at −80°C until analysis. Thawed samples were centrifuged for 5 min at 15,500 *g* and 4°C in a microcentrifuge to remove the cells and analysed light protected by HPLC. Subsequently, they were processed with a commercially obtained vitamin C analysis kit (Chromsystems Instruments & Chemicals GmbH, Vitamin C Diagnostics Kit by HPLC, Munich, Germany). The concentration of vitamin C in plasma, PMNs and PBMCs was determined by isocratic HPLC with UV detection and calculated according to the manufacturer's instructions. The following instrument settings had been used: injection volume 20 μl, run time 6–15 min., flow rate 1.3–1.5 ml/min., column temperature approximately 25°C and UV detector's wavelength 245 nm. Cellular content of vitamin C is expressed as $\mu g/10^8$ cells and plasma concentrations as mg/l. Plasma vitamin C levels were described as deficient (<2 mg/l), depleted (2–3.9 mg/l) and normal/saturated ($\geq$4 mg/l) according to internationally established limits (Loria et al. 1998). Subjects were, respectively, classified into three categories: deficient, depleted and normal.

Statistical analysis

Descriptive statistics and data analysis were performed with statistical software from SPSS (version 18.0 for Windows; Chicago, IL, USA). Differences between patients and controls and between vitamin C depleted and non-depleted patients were analysed using parametric or non-parametric tests, where appropriate. For continuous variables the data were first analysed whether they showed normal distributions (Kolmogorov–Smirnov goodness-of-fit test $p < 0.05$). For the non-normal distributed variables, log-transformed values were employed. Analysis of vitamin C concentration in leucocytes was based on 20 patients and 21 controls (data of one female non-smoking patient were missing due to laboratory technical problems). To assess the relationship between vitamin C concentrations in plasma and the concentrations in PMNs and PBMCs a regression analysis (backward stepwise linear regression with $p = 0.05$ to enter and $p = 0.10$ to leave) was employed with the number of cells as confounder where appropriate. To determine which variables to enter, we first calculated in both the control as well as the patient group individual correlations between vitamin C concentrations in plasma, PMNs, PBMCs on one hand and predictor variables on the other. The following predictor variables were tested: total intake of vitamin C, age, gender, ethnicity, smoking, education level, BMI, BOP and PPD (the latter two only in the patient group since they were not assessed in the control group). Next, in the control and patient group only for the predictors that showed a statistical significant correlation a regression analysis was run. For all analyses, $p < 0.05$ values were considered statistically significant.

Results

The background characteristics of the study population are presented in Table 1. No significant differences were observed between the two groups in relation to education, BMI, supplement use and number of teeth present. However, the numbers of PMNs and PBMCs were significantly higher in the patient group. Patients with moderate to severe periodontal destruction presented on average 8.4 teeth with $\geq$30% bone loss and 3.9 teeth with $\geq$50% bone loss.

The frequency of servings containing various amounts of vitamin C and the total calculated intake of vitamin C was not different between patients and controls (Table 2). However, in the control group females showed a higher total vitamin C intake than males i.e. 18.6 *versus* 9.9 servings; this phenomenon was not present in the patient group (Table 3). The mean values of plasma and cellular vitamin C concentrations are presented in Table 2. The plasma vitamin C concentration of the patients ranged from 2.44 to 15.99 mg/l and was significantly lower than those of the controls, which ranged from 3.12 to 18.2 mg/l. In the control group one subject showed vitamin C depletion (<4 mg/l), whereas this amounted to four subjects in the patient group. Vitamin C deficiency was not found in any of the subjects of the current study population. Female controls showed higher plasma vitamin C levels compared with male controls and female patients (Table 3). No gender effects were present in relation to the vitamin C levels in PMNs and PBMCs in both patients and controls.

The vitamin C levels in the PMNs ranged in the patient group between 12.5 and 124.0 $\mu g/10^8$ cells and in the controls between 34.8 and 97.2 $\mu g/10^8$ cells. Analysis revealed no statistically significant differences (Table 2). Also intracellular vitamin C of the PBMCs was not significantly different between patients and controls (Table 2); these values ranged from 15.2 to 200.3 $\mu g/10^8$ cells vitamin C in the patients, and from 56.5 to 151.3 $\mu g/10^8$ cells in the controls. In the control group, smok-

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Table 1. General characteristics of the study population. Values represent numbers (%) of subjects or means ± SD

Background characteristics	Control subjects (N = 21)	Periodontitis patients (N = 21)
Age (years)	46.4 ± 9.0	46.9 ± 10.8
Gender		
Male	10 (48%)	10 (48%)
Female	11 (52%)	11 (52%)
Ethnicity		
Non-Caucasian	4 (19%)	4 (19%)
Caucasian	17 (81%)	17 (81%)
Smoking		
Non-smoker	10 (48%)	10 (48%)
Smoker	11 (52%)	11 (52%)
Pack-year	20.1 ± 11.8	20.2 ± 13.8
Education		
<High school	4 (19%)	8 (38%)
≥ High school	17 (81%)	13 (62%)
Body mass index (kg/m^2)	24.1 ± 2.9	25.5 ± 3.4
Supplement use		
Vitamin C	2 (10%)	3 (14%)
Other	1 (5%)	2 (10%)
None	18 (85%)	16 (76%)
No. Leucocytes (10^9/l)		
PMNs	4.25 (1.69)	4.52 (2.03)*
PBMCs	2.24 (1.04)	2.67 (0.94)*
Clinical characteristics		
Number of teeth	26.7 ± 3.1	26.6 ± 2.7
% Bleeding on probing	–	70.2 ± 12.7
Probing pocket depth (mm)	–	3.7 ± 0.6
% Pockets ≥ 5 mm	–	26.7 ± 16.0
CAL (mm)	–	3.8 ± 1.1
% Of sites CAL ≥ 5 mm	–	29.1 ± 21.5
% Of teeth bone loss ≥ 30%	–	32.4 ± 15.9
% Of teeth bone loss ≥ 50%	–	15.4 ± 16.2

*$p < 0.001$.
For the non-matched background characteristics and number of teeth, no significant differences were found.
CAL, clinical attachment loss.

Table 2. Vitamin C intake and vitamin C concentration in plasma, PMNs and PBMCs for controls and periodontitis patients. Values are means (±SD)

	Control (N = 21)	Periodontitis (N = 21)	p-value
No. servings >31 mg/100 mg vitamin C	1.9 ± 2.0	1.1 ± 1.3	0.12
2–30 mg/100 mg vitamin C	10.2 ± 6.4	9.5 ± 4.1	0.68
<2 mg/100 mg vitamin C	29.1 ± 11.5	29.4 ± 9.4	0.92
Total intake vitamin C*	14.5 ± 9.8	12.3 ± 5.8	0.39
Vitamin C in plasma (mg/l)	11.3 ± 5.2	8.3 ± 3.9	0.03
Vitamin C in PMNs (μg/10^8 cells)	53.6 ± 14.7	61.6 ± 22.0†	0.18
Vitamin C in PBMCs (μg/10^8 cells)	90.9 ± 20.4	100.6 ± 50.8†	0.42

*Calculated as the number of servings containing 2–30 mg/100 mg vitamin C plus 2 × the number of servings containing ≥ 31 mg/100 mg vitamin C (including supplements) during the 3 days prior to the examination.
†N = 20.

ers had lower levels of vitamin C in PBMCs compared with non-smokers, i.e. 81.8 *versus* 100.9 μg/10^8 cells (Table 3). With regards to the relationship between plasma vitamin C levels and the vitamin C levels in PMNs and PBMCs in both the control and the patient group, no significant correlations were found.

The results of the relationship between the vitamin C concentrations in plasma, PMNs and PBMCs on one hand and the predictors on the other are presented in Table 4. In the control group a significant positive correlation was present between plasma levels and the total intake of vitamin C. In contrast, in the patient group none of the predictors showed a significant relationship with the plasma vitamin C values. For PMNs, only in the patient group a significant result was found i.e. PPD was negatively correlated with vitamin C in PMNs. For PBMCs, in the control group a negative correlation was found with smoking, i.e. non-smokers had higher vitamin C concentration in the PBMCs compared with smokers. A positive correlation was present between age and vitamin C in PBMCs in the patients.

The background and clinical characteristics of the vitamin C depleted and non-depleted patients are presented in Table 5. The mean values of the clinical periodontal parameters appeared to be all lower in the non-depleted compared with the depleted patients. However, only in case of the percentage of pockets 4–6 mm and the percentage of teeth with bone loss ≥ 50%, the difference reached level of significance. All four depleted patients were smokers whereas in the non-depleted group only seven (41%) of the patients were smokers. It is interesting to note that the study population of the patients included two subjects with far more periodontal bone loss than the others, i.e. number of teeth with bone loss >50%: 54% *versus* 11% respectively (p = 0.001). Both were smokers and showed vitamin C depletion i.e. the lowest plasma vitamin C values of all subjects of the study population: 2.4 and 2.6 mg/l. One of them showed an extremely low vitamin C concentration in the PBMCs (15.2 μg/10^8 cells), but normal concentrations in the PMNs. In the other patient the vitamin C concentrations in PMNs and PBMCs were within the normal range. Both patients had an average total vitamin C intake.

Discussion

The present study was initiated to investigate whether periodontitis is not only associated with lower plasma vitamin C levels but also with lower vitamin C intake and lower intracellular

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Table 3. Vitamin C intake and vitamin C concentrations in plasma, PMNs and PBMCs for controls (N = 21) and periodontitis patients (N = 21) in relation to gender (11 females), and smoking (10 non-smokers). Values are means (±SD)

		Control	Periodontitis	*p*-value
Total intake vitamin C				
Gender	Males	9.9 ± 7.8	10.6 ± 5.3	0.82
	Females	18.6 ± 9.8	14.0 ± 6.0	0.21
	p-value	0.037	0.20	
Smoking	Non-smokers	18.3 ± 10.7	13.3 ± 6.3	0.22
	Smokers	11.0 ± 7.7	11.3 ± 5.3	0.92
	p-value	0.087	0.45	
Vitamin C in plasma (mg/l)				
Gender	Males	9.1 ± 3.4	8.2 ± 3.8	0.58
	Females	12.6 ± 3.2	8.4 ± 3.9	0.012
	p-value	0.025	0.90	
Smoking	Non-smokers	11.6 ± 3.1	9.1 ± 2.6	0.067
	Smokers	10.4 ± 4.2	7.6 ± 4.6	0.16
	p-value	0.48	0.39	
Vitamin C in PMNs (μg/10^8 cells)				
Gender	Males	51.8 ± 13.0	55.2 ± 20.1	0.66
	Females	55.3 ± 16.5	67.9 ± 21.0*	0.16
	p-value	0.60	0.20	
Smoking	Non-smokers	56.6 ± 17.9	70.9 ± 22.7†	0.14
	Smokers	50.9 ± 11.2	53.9 ± 19.2	0.66
	p-value	0.39	0.087	
Vitamin C in PBMCs (μg/10^8 cells)				
Gender	Males	85.9 ± 14.0	100.3 ± 39.3	0.29
	Females	95.5 ± 24.6	100.9 ± 62.5*	0.79
	p-value	0.29	0.98	
Smoking	Non-smokers	100.9 ± 23.8	96.2 ± 59.0†	0.81
	Smokers	81.8 ± 11.3	104.2 ± 45.7	0.13
	p-value	0.027	0.74	

*N = 10.
†N = 9.

Table 4. Relationship between vitamin C intake, subjects characteristics and vitamin C concentrations in plasma, PMNs and PBMCs. Results of regression analyses of predictor variables that showed significant correlations

Predictor variables		β (95% CI)	Explained variance	*p*-value
Vitamin C concentration in plasma				
Controls	Total intake vitamin C	0.62 (0.94, 0.38)	38.4%	0.002
	Gender	n.r.	–	–
Patients	No predictors to enter into the model	–	–	–
Vitamin C concentration in PMNs				
Controls	No predictors to enter into the model	–	–	–
Patients	Pocket depth (mm)	−0.47 (−30.30, −0.90)	22.1%	0.039
	Bleeding on probing	n.r.	–	–
Vitamin C concentration in PBMCs				
Controls	Smoking*	−0.48 (−35.91, −2.38)	23.0%	0.027
Patients	Age	0.47 (0.15, 4.99)	22.1%	0.039
	Education	n.r.	–	–

*Non-smokers have higher vitamin C concentrations than smokers.
n.r.: not retained in the model.

stores of vitamin C in leucocytes to explain a possible mechanism of this micronutrient in the pathogenesis of periodontitis. The present findings confirm previous results that lower plasma vitamin C concentrations are associated with periodontitis (Melnick et al. 1988, Panjamurthy et al. 2005, Staudte et al. 2005, Thomas et al. 2010). The mean plasma vitamin C levels of the controls in the present study (11.3 mg/l) are comparable to figures from controls in case-control studies of the medical literature which range from 11.5 mg/l to 13.4 mg/l (Göçmen et al. 2008, Hengstermann et al. 2008, Braga et al. 2011, Chen et al. 2011, Zanon-Moreno et al. 2011). In the periodontitis group of our study the mean plasma vitamin C level amounted to 8.3 ± 3.9 mg/l. This value is in accordance with data from studies where vitamin C in plasma/serum was measured in periodontitis patients using HPLC: 7.9 ± 5.4 mg/l (Amaliya et al. 2007) and 7.2 mg/l (range 0.2–22.6 mg/l) (Amarasena et al. 2005).

The results of the multiple regression analysis showed that in the control group a highly significant correlation existed between the plasma vitamin C values and the total vitamin C intake whereas such a relationship was not present in the patient group. The finding that in disease, the vitamin C intake does not correspond to plasma vitamin C levels is not a new observation. For example, it has been shown that in *Helicobacter pylori* infections, the plasma vitamin C levels are lower than expected on the basis of the vitamin C intake (Woodward et al. 2001). This could be explained by an increased turnover of vitamin C due to ROS generated by the inflammatory response. It has been shown that low vitamin C plasma values are related to disease conditions that are thought to be caused or exacerbated by oxidative stress. However, it is not clear whether low plasma and tissue vitamin C levels either contribute to each of these diseases or are a consequence of the disease process or merely associated with the disease condition (Padayatty et al. 2003). Another explanation for the absence of a positive correlation between vitamin C intake and plasma vitamin C values in periodontal patients may be a different genetic make-up. Vitamin C can be actively transported across membranes against its concentration gradient by two sodium-dependent vitamin C transporter proteins (Stratakis et al. 2000) encoded by two separate genes and for both genetic polymorphisms have been demonstrated (Eck et al. 2004). In a recent study, it was confirmed that genetic variation in vitamin C trans-

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Table 5. Background and dental characteristics (mean ± SD) of vitamin C depleted and non-depleted periodontitis patients. Values represent numbers (%) of subjects or means ± SD

	Patients by vitamin C group		
	Depleted (N = 4)	Non-depleted (N = 17)	p-value
Background characteristics			
Age (years)	44.5 ± 3.9	47.5 ± 11.8	0.63
Gender (males)	2 (50%)	8 (47%)	1.00
Ethnicity (Caucasians)	4 (100%)	13 (77%)	0.55
Smoking (smokers)	4 (100%)	7 (41%)	0.09
Education (<high school)	1 (25%)	8 (47%)	1.00
Body mass index (kg/m^2)	23.1 ± 0.7	26.1 ± 3.6	0.12
Supplement use (none)	4 (100%)	12 (71%)	0.53
Dental characteristics			
Number of teeth	26.5 ± 1.9	26.5 ± 2.8	0.92
Bleeding on probing (%)	71.5 ± 12.3	69.9 ± 13.1	0.82
Probing pocket depth (mm)	4.0 ± 0.6	3.6 ± 6.6	0.25
% Pockets 4–6 mm	45.7 ± 6.6	33.2 ± 11.4	0.05
CAL (mm)	4.4 ± 1.7	3.6 ± 0.9	0.15
% Sites CAL $\geq$5 mm	40.8 ± 31.6	26.3 ± 18.6	0.24
% Teeth with bone loss $\geq$30%	9.3 ± 1.0	8.2 ± 4.3	0.65
% Teeth with bone loss $\geq$50%	7.8 ± 7.3	3.1 ± 2.5	0.03

CAL, clinical attachment loss.

porter protein SVCT1 can influence serum vitamin C concentrations and that different SVCT1 and SVCT2 genotypes may influence vitamin C uptake and therefore modify the strength of the correlation between dietary and serum vitamin C (Cahill & El-Sohemy 2011).

The PMNs and PBMCs are able to accumulate vitamin C and may contain 10–40 times higher concentrations than plasma (Oberritter et al. 1986), a phenomenon that was confirmed in the present study. It has been suggested that high vitamin C levels in leucocytes contribute to the ability of these cells to react to inflammatory stimuli (Boxer et al. 1979). Several studies have shown that putative periodontal pathogenic bacteria elicit ROS from PMNs and that in the absence of suitable antioxidants ROS derived from PMNs can result in tissue damage (Kimura et al. 1993, Waddington et al. 2000). In line with this observation is the negative association between PPD and vitamin C concentration in PMNs of the periodontitis patients of the present study. This could be explained by a higher turnover of vitamin C in PMNs of patients with a higher periodontal inflammatory burden.

The hypothesis of the present study was that in periodontitis vitamin C concentration in leucocytes could be reduced. However, no differences were found between patients and controls regarding the vitamin C levels in PMNs and PBMCs. As mentioned before, the phenomenon that presence of disease results in lower vitamin C concentration in leucocytes has been reported in asthmatic (Shidfar et al. 2005) and in diabetes patients (Cunningham et al. 1991, Akkuş et al. 1996). One explanation for the lack of reduced concentrations of vitamin C in leucocytes of periodontitis patients may be that periodontitis patients have higher levels of interleukin-8, granulocyte macrophage colony-stimulating factor and interferon-α in the circulation. Dias et al. (2011) showed that this condition could contribute to the hyperactive PMN phenotype seen in periodontitis patients. The hyperactive PMNs may use an increased amount of intracellular vitamin C which is compensated by a higher uptake of vitamin C from the bloodstream, resulting in lower plasma vitamin C levels and normal levels in the PMNs. However, another explanation may be the specific situation of the periodontal tissues. Within the periodontal tissues a local oxidative stress situation has been demonstrated, as evidenced by a compromised antioxidant status of the gingival crevicular fluid (Chapple et al. 2002, Brock et al. 2004). It may be suggested that this compromised status could be due to a reduced concentration of vitamin C in the PMNs of the crevicular fluid. These PMNs with possibly low intracellular vitamin C stores do not return into the blood circulation but are leaving the tissues into the oral cavity. It has been estimated that the number of leucocytes leaving the body in this way amounted to 30,000 PMNs per min. in gingivitis (Schiött & Löe 1970) and this may be even higher in periodontitis. Consequently, the majority of the PMNs with a reduced vitamin C concentration do not contribute to the PMNs of the circulation of periodontitis patients and therefore the vitamin C concentration of PMNs remains within normal limits.

In conclusion, the results of the present research study as well as of previous investigations demonstrated that lower plasma vitamin C concentrations are associated with periodontitis. In the present small patient sample, those who showed vitamin C depletion had more bone loss compared with the non-depleted patients. This corroborates with the significant inverse association found between the plasma vitamin C levels and the severity of periodontitis in a relatively large population deprived from regular dental care in a small Indonesian village (Amaliya et al. 2007) as well as in an elderly Japanese population (Amarasena et al. 2005). Nevertheless, no differences could be assessed between the vitamin C concentrations in leucocytes of periodontitis patients and periodontally healthy controls. Therefore, the relationship between low plasma vitamin C levels and periodontitis cannot be explained by insufficient vitamin C storage capacity of the leucocytes and the question remains through which mechanism low plasma vitamin C levels are related to the development and/or severity of periodontitis.

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Clinical Relevance

Scientific rationale for the study: During the last decades it became evident that periodontitis is associated with low plasma vitamin C levels. There is also growing evidence for the importance of vitamin C for leucocyte function and as antioxidant against reactive oxygen species (ROS) in periodontitis. However, there are currently no data available of vitamin C concentrations in PMNs and PBMCs of periodontitis patients.

Principal findings: Vitamin C intake and vitamin C concentrations in PMNs and PBMCs were not different between patients and controls. However, plasma vitamin C was reduced in periodontitis patients compared with controls. The positive relationship between vitamin C intake and plasma concentration in controls was not present in periodontitis patients. In periodontitis, increasing probing pocket depth was correlated with decreasing vitamin C concentrations in PMNs.

Practical implications: Although the relationship between low plasma vitamin C concentrations and periodontitis could not be explained by a hampered leucocyte function due to a lack of intracellular vitamin C, it seems justified that in periodontal practice more attention is paid to the diet of patients in relation to vitamin C.

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