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Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis

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Abstract

Objective: To study the relationship between vitamin C and the severity of periodontitis.

Material and Methods: The study population consisted of subjects from the Malabar/Purbasari tea estate on West Java, Indonesia. In 2002, clinical measurements were performed in 128 subjects, including evaluation of plaque, bleeding on probing, pocket depth and attachment loss. In 2005, 123 out of 128 subjects could be retrieved who were present at the examination of 2002. Blood samples were taken to measure plasma vitamin C levels. Information about the subject's dietary habit was obtained by means of a personal interview guided by a questionnaire.

Results: Plasma levels of vitamin C ranged from 0.02 to 34.45 mg/l with a mean of 7.90 mg/l ($\pm$ 5.35). The correlation coefficient between plasma vitamin C level and periodontal attachment loss was -0.199 ($p<0.05$); stepwise linear regression revealed that vitamin C levels explained 3.9% of the variance in periodontal attachment loss. Subjects with vitamin C deficiency (14.7% of the study population) had more attachment loss compared with those with depletion or normal plasma vitamin C values.

Conclusion: The negative association between plasma vitamin C levels and periodontal attachment loss suggests that vitamin C deficiency may contribute to the severity of periodontal breakdown.

Key words: periodontitis; untreated disease; vitamin C

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At present, it is well accepted that periodontitis is a multifactorial disease caused by an imbalance between environmental factors such as periodontal pathogens, and the host defence. Host–defence mechanisms may be influenced by genetic factors, hormones and nutrition. With regard to the latter, especially vitamin C has received considerable attention in the periodontal literature as absolute deficiency results in the clinical condition known as scurvy (Woolfe et al. 1980). In addition, a strong relationship between vitamin C deficiency and acute necrotizing ulcerative gingivitis (ANUG) has frequently been described. For example, Melnick et al. (1988) showed in a large case–control study that patients with a history of ANUG ingested less vitamin C as compared with the healthy control group.

Although early studies in animals showed that vitamin C deficiency leads to deeper pockets and increased periodontal breakdown (Glickman 1948a, b), the majority of the early epidemiological studies found no relationship between plasma vitamin C levels and the degree of periodontal disease (Burrill 1942, Barros & Witkop 1963, Russell 1963, Russell et al. 1965, Enwonwu & Edozien 1970). In contrast, more recent epidemiological studies have shown a significant relationship between vitamin C and the periodontal condition. Vogel & Wechsler (1979) found that

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the daily intake of vitamin C in a group of periodontitis patients was significantly less than that in the control subjects. On the basis of the NHANES I study, Ismail et al. (1983) found a weak but significant correlation between dietary vitamin C intake and periodontal disease after controlling for potentially confounding variables of age, gender, race, education, income and oral hygiene status. In South Africa, Blignaut & Grobler (1992) observed pockets $\geqslant$4 mm less frequently in citric fruit farm workers who consumed large amounts of fruit compared with grain farm workers who did not. In a case–control study matched for age, sex and number of teeth, Vaananen et al. (1993) studied the periodontal condition in subjects with low ($\leqslant$4.4 mg/l) and high ($\geqslant$8.8 mg/l) plasma vitamin C levels. In the group with low plasma vitamin C levels, 60% of the subjects had pockets $\geqslant$4 mm compared with 37% in the group with high plasma vitamin C levels. On the basis of the NHANES III survey, Nishida et al. (2000) found that the dietary intake of vitamin C showed a weak, but statistically significant relationship with periodontal disease in current and former smokers. Smokers taking the lowest intake of vitamin C are likely to have the worst periodontal condition. In a recent study, Amarasena et al. (2005) showed in an elderly population an inverse relationship between serum vitamin C levels and attachment loss irrespective of smoking, diabetes, oral hygiene, gender, or the number of teeth present. The above-reviewed literature suggests that insufficient intake of vitamin C could aggravate the progression of periodontal breakdown.

Recently, the results of the 15-year longitudinal Java project on periodontal diseases became available, which evaluated the initiation and progression of periodontal disease in an Indonesian rural population deprived from regular dental care (Van der Velden et al. 2006). The results showed that 20% of the population developed severe periodontitis. Unfortunately, at the start of the project, evaluation of nutritional aspects was not included in the study protocol although it is not unlikely that in this rural area the vitamin C intake may be low. Therefore, the aim of the present investigation was to study in this population the relationship between vitamin C, as assessed by plasma vitamin C level and dietary habits, and the severity of periodontitis.

Material and Methods

The design of the investigation and study population from the Malabar/Purbasari tea estate on West Java has been described in detail in the most recent report of this longitudinal study (Van der Velden et al. 2006). At baseline in 1987, all subjects of 15–25 years age of one village were included in the study. In 2002, 128 subjects could be retrieved out of the 255 subjects originally evaluated in 1987. For the present study, the data of the 15-year evaluation in 2002 were used. In short, subjects were asked about their education level, general health status, recent use of antibiotics and smoking habits in terms of number of cigarettes per day. Clinical evaluation included assessment of plaque (Silness & Löe 1964), bleeding on probing (Van der Velden 1979), pocket depth and attachment loss. These measurements were recorded at all approximal surfaces from the vestibular aspects. The reproducibility of these measurements is reported in a previous publication (Van der Velden et al. 2006). For the present study in 2005, 123 subjects could be retrieved out of the 128 subjects evaluated in 2002.

After identification of each subject, non-fasting venous blood samples were collected by the local hospital staff of the tea estate in vacuum tubes containing lithium heparin. After collection, whole-blood samples were centrifuged with a low-speed centrifuge (Shanghai surgical instrument factory, Shanghai, China) at 4000 r.p.m. for 4 min. to separate plasma from blood cells. To minimize the oxidation of the vitamin C, the latter procedure was performed within 10 min. after sampling. Vitamin C in heparin plasma is stable at room temperature up to 2 h. The plasma obtained was subsequently subjected to the preparation procedures according to the instruction manual for Chromsystems High-pressure liquid chromatography (HPLC)-Analysis of vitamin C in plasma (Chromsystems, Vitamin C diagnostics kit by HPLC, Munich, Germany). In a light-protected microreaction vial, 100 μl of the reconstituted precipitation reagent that contained the internal standard was pipetted and 100 μl of either standard, control or specimen plasma was added. Vials were vortexed for 10 s. The mixtures were incubated for 10 min. at +4°C and centrifuged for 5 min. at 13,000 r.p.m. in a microcentrifuge (Heraeus Biofuge Fresco, Hanau, Germany). The supernatants obtained from these procedures were kept in the refrigerator at 4°C until transportation to the laboratory in Bandung. According to the manual of the manufacturer, under these conditions, vitamin C is stable at 4°C for 4 days and at $-$20°C for at least 4 weeks. All samples were collected within 3 days and transported early in the morning the next day, within 4 h, on ice to the laboratory in Bandung. In the laboratory, the samples were stored at $-$20°C and analysed during the subsequent days.

Plasma vitamin C levels were determined by means of HPLC. The analysis of vitamin C requires a simple, isocratic system with an HPLC pump, an injector and a UV detector. The HPLC instrument used in this study was set with the following specifications: an injection volume of 20 μl, a run time of 5 min., a flow rate at 1–1.5 ml/min., a column temperature of approximately 25°C and the UV detector's wavelength at 245 nm (Hewlett Packard HPLC Instrument, HP-1100, ON, Canada). The concentration of vitamin C in the sample was calculated according to the manufacturer's instructions. Plasma vitamin C levels were categorized according to internationally established limits: deficiency (less than 2 mg/l), depletion (2–3.9 mg/l) and normal (4.0 mg/l or more; Hampl et al. 2004).

Information about the subject's dietary habits during the last month was obtained by means of a personal interview guided by a questionnaire that had been developed in advance. In addition, the subjects were asked which nutrients they had consumed on the day of the examination before the blood samples were taken. The level of vitamin C content of the various nutrients was based on the values provided by the Indonesian Nutritional Network (2006).

Statistical analysis

The clinical parameters at the 2002 follow-up assessment were calculated as mean scores per patient. The mean clinical parameters, the mean plasma vitamin C levels and the mean frequencies of monthly dietary intake were calculated for each category of plasma vitamin C level. To compare differences between means, Student's *t*-test and one-way ANOVA were used when appropriate. Stepwise multiple linear regression analysis was used to test for a possible association between plasma vitamin C level and the amount of attachment loss as found in 2002. Mean attachment loss was entered as the dependent variable and plasma

vitamin C was entered as the obligatory independent variable in the first layer of the model, whereas age, gender, smoking status, education level and plaque were entered as independent variables in the second, stepwise layer of the model. *p*-values of <0.05 were accepted as being statistically significant.

Table 1. Significant variables for severity of attachment loss in 2002 as assessed by means of a multiple linear stepwise regression analysis ($N = 123$)

Variable	Unstandardized coefficient *B*	SE	Correlation coefficient β	*p*-value	% explained variance
Plasma vitamin C level	− 0.042	0.019	− 0.199	0.029	3.9
Model	− 0.312	1.437		<0.0001	

SE, standard error.

Results

The 123 subjects who participated in this study included 64 females and 59 males with an age range of 33–43 years. Fifty-three subjects were smokers: 52 males and one female. In general, subjects exhibited a low education level; 60 subjects had completed elementary school, whereas 63 had not. The mean plaque index, bleeding on probing, pocket depth and attachment loss as assessed in 2002 in these subjects were 1.05 ($\pm$ 0.40), 1.22 ($\pm$ 0.39), 3.53 mm ($\pm$ 0.58) and 3.63 ($\pm$ 8.38), respectively.

Plasma levels of vitamin C ranged from 0.02 to 34.45 mg/l with a mean of 7.90 mg/l ($\pm$ 5.35). 71.5% of the study population had normal plasma vitamin C levels, whereas 13.8% showed depletion and 14.7% deficiency for vitamin C. No statistically significant differences in plasma vitamin C values were found with regard to smoking [smokers *versus* non-smokers: 6.90 ($\pm$ 4.62) and 8.29 ($\pm$ 5.50) mg/l, respectively, $p = 0.14$] as well as gender (male *versus* female: 6.97 ($\pm$ 4.49) and 8.35 ($\pm$ 5.67) mg/l, respectively, $p = 0.14$). Among males, 16.9% were deficient for vitamin C, whereas 12.5% of females showed deficiency.

In order to determine a possible correlation between plasma vitamin C levels and the severity of attachment loss in 2002, a stepwise multiple linear regression analysis was carried out including the variables age, gender, smoking and plaque. The results revealed that only vitamin C showed a statistically significant relationship with the amount of attachment loss. Lower plasma vitamin C levels were related to more periodontal breakdown; vitamin C levels explained 3.9% of the variance (Table 1). The age and periodontal characteristics of the subjects, as assessed in 2002, in relation to their plasma vitamin C status are presented in Table 2. Analysis showed no differences between the three vitamin C categories except for attachment loss. Post-hoc testing showed that the group that was deficient for vitamin C had significantly more attachment loss compared with the normal or depletion group.

Table 2. Age and periodontal characteristics of the study population by vitamin C category

Vitamin C (mg/l)	Deficiency	Depletion	Normal
N	< 2.0 mg/l	2.0–3.9 mg/l	⩾4 mg/l
	18	17	88
Age (years)	36.6 (2.9)	36.5 (3.6)	35.7 (3.1)
PI	1.08 (0.47)	0.99 (0.32)	1.05 (0.41)
PD	3.60 (0.59)	3.61 (0.73)	3.50 (0.55)
BOP	1.25 (0.48)	1.24 (0.41)	1.22 (0.37)
AL*	2.58 (1.12)	2.11 (1.23)	1.83 (1.04)

N, number of subjects; PI, plaque index; PD, probing depth; BOP, bleeding on probing; AL, attachment loss; standard deviation between parentheses.
*ANOVA, $p = 0.025$.

The mean frequency of monthly food intake by level of vitamin C content in relation to the plasma vitamin C level category is shown in Table 3. Food containing less than 2 mg vitamin C/100 g was classified as no vitamin C, 2–20 mg vitamin C/100 g as low amounts, 21–60 mg vitamin C/100 g as fair and 61–280 mg/100 g as sources of high vitamin C content. No statistically significant differences could be assessed in the frequency of food intake regarding vitamin C between the three plasma vitamin C level categories. In Table 4 data are presented regarding the nutrition and level of content of vitamin C that was consumed on the day of examination before the blood samples were taken. It can be seen that in all three plasma vitamin C categories, the majority of subjects had consumed chili. Only the percentage of subjects who had consumed kangkung (a kind of vegetable) was higher in the groups with vitamin C levels above 4.0 mg/l. However, the monthly intake of kangkung was not different between those who had consumed kangkung on the day of examination and those who had not; the mean number of times per month being 4.09 $\pm$ 7.3 and 3.82 $\pm$ 8.8, respectively.

Table 3. Mean frequency of vitamin C intake per month by nutrient and level of content

N	Deficiency 18	Depletion 17	Normal 88
High vitamin C: 61–280 mg/100 g			
Cassava leaves	3.83	4.06	4.81
Kangkung	2.11	5.29	3.92
Chili	28.89	24.92	27.55
Guava	11.17	11.00	11.48
Orange	2.17	1.18	0.88
Fair vitamin C: 21–60 mg/100 g			
Sweet potato	2.67	5.06	4.40
Low vitamin C: 2–20 mg/100 g			
Cabbage	9.72	11.29	11.42
Toge	2.72	5.65	5.61
Banana	4.17	8.71	6.89
Avocado	1.11	2.12	1.90
Carrot	9.89	6.24	10.06
Potato	1.11	1.88	3.44
Onion	28.89	24.82	28.88
No vitamin C: <2 mg/100 g			
Cassava	3.94	8.47	6.20
Corn	1.17	1.06	1.66
Rice	45.67	52.47	62.59
Tofu	20.11	16.18	17.43
Tempe	9.11	10.53	16.05
Red bean	1.61	3.47	2.28
Salted fish	3.94	9.24	6.17
Carp	2.00	3.35	2.27
Goldenfish	3.11	2.12	3.41
Chicken	2.44	3.06	2.93
Lamb	0.00	0.18	0.20
Beef	1.17	0.76	1.14
Egg	13.00	17.53	16.53
Garlic	28.89	27.41	27.41

N, number of subjects.

Discussion

The results of the present study revealed a small but statistically significant

Table 4. Number (%) of subjects by nutrition that they consumed on the day of examination before blood sampling

N	Deficiency 18	Depletion 17	Normal 88
High vitamin C: 61–280 mg/100 g			
Cassava leaves	0	0	2 (2.2)
Kangkung*	0	0	15 (17.0)
Chili	13 (72.2)	10 (58.8)	53 (60.2)
Guave	0	0	0
Orange	1 (5.6)	0	0
Fair vitamin C: 21–60 mg/100 g			
Sweet potato	1 (5.6)	0	0
Low vitamin C: 2–20 mg/100 g			
Cabbage	7 (38.9)	3 (17.6)	39 (44.3)
Toge	2 (11.1)	1 (5.9)	4 (4.5)
Banana	0	0	0
Avocado	0	0	0
Carrot	2 (11.1)	1 (5.9)	10 (11.4)
Potato*	0	0	15 (17.0)
Onion	11 (61.1)	9 (52.9)	40 (45.6)

N, number of subjects; *ANOVA, $p = 0.03$.

inverse association between the plasma vitamin C levels and the severity of periodontitis as assessed by mean attachment level obtained from the measurements in 2002. The negative correlation between plasma vitamin C levels with the severity of attachment loss could mainly be explained by the finding that subjects with vitamin C deficiency had more attachment loss compared with those with depletion or normal plasma vitamin C values.

Although the plasma vitamin C was determined in 2005, does not it seem likely that the periodontal condition had changed much during this 3–year interval, as subjects were still deprived from regular dental care. At the very most, attachment loss could have progressed to some extent. It is also unlikely that the dietary habits of this population had changed much during this 3-year period. The present results with regard to vitamin C are in agreement with the findings of the epidemiological studies of the last decades investigating the relationship between vitamin C and periodontal disease (Ismail et al. 1983, Blignaut & Grobler 1992, Vaananen et al. 1993, Nishida et al. 2000, Amarasena et al. 2005). There are several plausible biological explanations regarding how vitamin C could affect the periodontal tissues. For example, vitamin C deficiency may result in a lack of collagen formation (Berg et al. 1983), increased permeability of gingival mucosa (Alfano et al. 1975, Alvares & Siegel 1981) and reduced neutrophil function (Washko et al. 1991).

The discussion about the role of vitamin C in periodontal disease is hindered by the results of studies that have investigated the role of vitamin C supplementation. Parfitt & Hand (1963) found that a daily dose of 500 mg vitamin C had no effect on gingival health despite the fact that at the start of the study, the gingival health of the subjects was poor and the plasma vitamin C levels were low. No effect of vitamin C supplementation on the development of experimental gingivitis was found by Vogel et al. (1986) when a daily dose of 1500 mg was consumed. On the other hand, studies on experimental vitamin C depletion and supplementation showed a direct relationship between gingival inflammation and vitamin C status (Legott et al. 1986, Jacob et al. 1987). In a recent study, it was found that periodontitis patients are characterized by plasma vitamin C levels below the normal range and that grapefruit consumption reduced the sulcus bleeding scores but not the probing depth (Staudte et al. 2005). The improved gingival health as found in that study was most likely due to the vitamin C supplementation by means of grapefruits. Recent findings have also indicated that citrus fruits are more effective in increasing the plasma vitamin C levels as compared with high-dose supplements (Sánchez-Moreno et al. 2003).

In the present study, large variations were found with regard to the plasma vitamin C levels. It was surprising that this variation could not be explained by smoking because it has been found that cigarette smokers have lower plasma vitamin C values compared with non-smokers (Chow et al. 1986, Schectman et al. 1989). In addition, it has been shown that cigarette smokers have a higher turnover of vitamin C than non-smokers (Kallner et al. 1981). The lack of relationship between smoking and plasma vitamin C levels in the present study may be explained by the fact that the smokers in this population do not smoke normal cigarettes but kretek cigarettes. Kreteks are also known as clove cigarettes, as they typically contain 40% cloves and 60% tobacco. They are promoted as being less harmful (WHO, 2006). The fact that kretek smoking may have not the same effects as cigarette smoking seems to be supported by the finding that in this population, no difference in the amount of attachment loss could be found between smokers and non-smokers. The lack of relationship between smoking, gender and attachment loss in this population has been extensively discussed in a previous paper (Van der Velden et al. 2006).

It is well known that dietary intake of vitamin C is reflected in higher plasma vitamin C values. Fruit, vegetables and/or fruit juice three or more times a day increases plasma vitamin C levels above the threshold for risk of deficiency (Wrieden et al. 2000). This phenomenon is supported in the present population by the finding that those who had consumed kangkung on the day of the examination all had plasma vitamin C values above 6.5 mg/l. The finding that almost half of the study population had optimal plasma vitamin C values was surprising, as the vitamin C content of the food appears to be rather low and also the mean intake frequency per month of the vitamin C-containing nutrients is low. It is interesting to note that due to the cooking habits of this population, all vegetables undergo prolonged cooking, which probably also diminishes the vitamin C content of consumed food. The most important source of vitamin C in this population seems to be chili. This nutrient is consumed almost on a daily basis and contains high amounts of vitamin C. Unfortunately, the amount of chili consumed was not evaluated but it may be supposed that variations in the amount of chili consumed could have contributed to the observed variation in plasma vitamin C levels.

The fact that intake of vitamin C 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 also lower than expected on the basis of the vitamin C intake (Woodward et al. 2001). Park et al. (2003) found a negative correlation between vitamin C levels in the blood and the degree of active and chronic inflammation of the gastric mucosa. *Helicobacter pylori* has been shown to potentiate the polymorphonuclear leucocyte (PMN) oxidative burst, which is accompanied by a considerable production of reactive oxygen metabolites (Mooney et al. 1991). Park et al. (2003) suggested that vitamin C within the microcirculation of the gastric mucosa is used to scavenge the reactive oxygen metabolites from the PMN; vitamin C is considered the first line of defence against the oxygen free radical damage in the body. It may be hypothesized that a comparable mechanism occurs in the periodontal tissues. Hence, the individual variation in the extent of the periodontal infection could contribute to the differences in plasma vitamin C levels between subjects as found in the present study.

Another explanation could be that the bioavailability varies between subjects. Bioavailability is a measure of efficiency of gastrointestinal tract absorption of e.g. vitamin C. Vitamin C is directly transported across membranes by two sodium-dependent vitamin C transporter proteins (Stratakis et al. 2000) and for both genes, genetic variations have been demonstrated (Eck et al. 2004). Interestingly, in a recent study, genetic variations in these two genes were linked to preterm birth (Erichsen et al. 2006), a condition that has also been associated with periodontitis (Moliterno et al. 2005). Unfortunately, in these studies, the dietary vitamin C intake was not evaluated. In total, it does not seem unlikely that gene polymorphisms for the vitamin C transporter proteins could have contributed to the variations in vitamin C levels of the present population. This is part of further research.

In conclusion, the present study demonstrated that in this population, deprived from regular dental care, low plasma vitamin C levels were related to more periodontal attachment loss.

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Clinical Relevance

Scientific rationale for the study: Nutrient deficiency may have an influence on the severity of periodontitis. Insufficient vitamin C supply could aggravate the progression of periodontal breakdown.

Principal findings: The level of vitamin C in plasma was negatively correlated with the amount of periodontal attachment loss. The inverse association between plasma vitamin C levels and the amount of attachment loss could mainly be explained by the finding that subjects with vitamin C deficiency had more attachment loss compared with those with depletion or normal plasma vitamin C values.

Practical implications: When considering measures to improve periodontal health, the composition of the diet, with special attention to the content of vitamin C, may be an addition to the armamentarium of periodontal care.