Java project on periodontal disease. Periodontal condition in relation to vitamin C, systemic conditions and tooth loss

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Chapter 2

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 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 in 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 personal interview guided by questionnaire.

Results: Plasma levels of vitamin C ranged from 0.02 to 34.45 mg/l with a mean of 7.90 mg/l (± 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 to 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.

Introduction

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 defense. Host defense mechanisms may be influenced by genetic factors, hormones and nutrition. With regard to the latter, especially vitamin C has received much attention in the periodontal literature since 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 to 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, Russell 1963, Russell et al. 1965, Barros & Witkop 1963, 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 the daily intake of vitamin C in a group of periodontitis patients was significantly less than 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 $\geq$ 4 mm less frequently in citric fruit farm workers who consumed large amounts of fruit compared to grain farm workers who did not. In a case control study matched for age, sex and number of teeth, Väänänen et al. (1993) studied the periodontal condition in subjects with low ($\leq$ 4.4 mg/l) and high ($\geq$ 8.8 mg/l) plasma vitamin C levels. In the group with low plasma vitamin C levels, 60% of the subjects had pockets $\geq$ 4 mm compared to 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 to 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 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 became available of the 15-year longitudinal Java project on periodontal diseases, 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.

Materials and Methods

The design of the investigation and study population from the 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 aging 15-25 years of 1 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 rpm for 4 minutes to separate plasma from blood cells. To minimize the oxidation of the vitamin C the latter procedure was performed within 10 minutes after sampling. Vitamin C in heparin plasma is stable at room temperature up to 2 hours. The plasma obtained was subsequently subjected to the preparation procedures according to the instruction manual for Chromsystems HPLC-

Analysis of Vitamin C in plasma (Chromsystems, Vitamin C Diagnostics Kit by HPLC, Munich, Germany). In a light protected micro reaction vial, 100 µl of the reconstituted Precipitation Reagent that contained the Internal Standard was pipetted and 100 µl was added of either standard, control or specimen plasma. Vials were vortexed for 10 seconds. The mixtures were incubated for 10 minutes at + 4^{o} C and centrifuged for 5 minutes at 13 000 rpm in a micro centrifuge (Heraeus Biofuge Fresco, Hanau, Germany). The supernatants obtained from these procedures were kept in the refrigerator at 4^{o} C until transportation to the laboratory in Bandung. According to the manual of the manufacturer under these conditions vitamin C is stable at 4^{o} C for 4 days and at -20^{o} C for at least 4 weeks. All samples were collected within 3 days and early in the morning of the next day transported, within 4 hours, on ice to the laboratory in Bandung. In the laboratory the samples were stored at -20^{o} C and analysed during the subsequent days.

Plasma vitamin C levels were determined by means of High Pressure Liquid Chromatography (HPLC). The analysis of vitamin C requires a simple, isocratic system with an HPLC pump, injector and 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, column temperature approximately 25^{o}C, and the UV detector's wavelength at 245 nm (Hewlett Packard HPLC Instrument, HP-1100, Ontario, 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 nomal (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 which 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 food products was based on the values provided by the National Nutrient Database for Standard Reference (USDA 2010) and Woot-Tsuen et al. (1968).

Statistical Analysis

The clinical parameters at the 2002 follow-up assessment were calculated as mean scores per patient. Mean clinical parameters, mean plasma vitamin C levels and 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 employed

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 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 statistically significant.

Results

The 123 subjects that 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 1 female. In general, subjects exhibited a low education level, 60 subjects completed the elementary school whereas 63 did not. The mean plaque index, bleeding on probing, pocket depth and attachment loss as assessed in 2002 in these subjects were 1.05 (± 0.40), 1.22 (± 0.39), 3.53 mm (± 0.58) and 3.63 (± 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 (± 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 (± 4.62) and 8.29 (± 5.50) mg/l respectively, p=0.14) as well as to gender (male versus female: 6.97 (± 4.49) and 8.35 (± 5.67) mg/l respectively, p=0.14). Among males, 16.9% was 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 statistical 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).

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

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 3 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 to the normal or depletion group.

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

	Deficiency	Depletion	Normal
Vitamin C (mg/l)	< 2.0 mg/l	2.0-3.9 mg/l	≥ 4 mg/l
N	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 frequency of food intake regarding vitamin C between the 3 plasma vitamin C level categories.

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

	Deficiency	Depletion	Normal
N	18	17	88
High vitamin C 61-280 mg/100g			
Cassava leaves	3.83	4.06	4.81
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/100gr			
Kangkung	2.11	5.29	3.92
Sweet potato	2.67	5.06	4.40
Low vitamin C 2-20 mg/100 g			
Cabbage	9.72	11.29	11.42
Soybean sprout	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
Onion	28.89	24.82	28.88
Cassava	3.94	8.47	6.20
No vitamin C <2 mg/100 g			
Corn	1.17	1.06	1.66
Rice	45.67	52.47	62.59
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

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 3 plasma vitamin C categories the majority of subjects had consumed chili. Only the percentage of subjects that 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 that had consumed kangkung on the day of examination and those that had not; mean number of times per months 4.09 ± 7.3 and 3.82 ± 8.8 respectively.

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

	Deficiency	Depletion	Normal
N	18	17	88
High vitamin C			
61-280 mg/100g			
Cassava leaves	0	0	2 (2.2)
Kangkung*	0	0	15 (17.0)
Chili	13 (72.2)	10 (58.8)	53 (60.2)
Guava	0	0	0
Orange	1 (5.6)	0	0
Fair vitamin C			
21-60 mg/100g			
Sweet potato	1 (5.6)	0	0
Low vitamin C			
2-20 mg/100g			
Cabbage	7 (38.9)	3 (17.6)	39 (44.3)
Soybean sprouts	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

Discussion

The results of the present study revealed a small but statistically significant 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 to those with depletion or normal plasma vitamin C values.

Although the plasma vitamin C was determined in 2005 it seems not likely that the periodontal condition had changed much during this 3 years interval, since 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 have 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, Väänänen et al. 1993, Nishida et al. 2000, Amarasena et al. 2005). There are several plausible biological explanations 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 was poor and the plasma vitamin C level 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 to 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 to 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 explained that 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 tipically 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 amount of attachment loss could be assessed 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 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, since 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 consumed Chili was not evaluated but it may be supposed that variations in amount of consumed chili 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. H. pylori has been shown to potentiate the polymorphonuclear leukocyte (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 defense against the oxygen free radical damage in the body. It may be hypothesized that a comparable mechanism occurs in the periodontal tissues. So, 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 all it seems not 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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