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

Materials 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 (± 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.
**Introduction**

Today, it is well accepted that periodontitis is a multifactorial disease, dependent on the delicate balance between the exogenous factors influencing the oral environment and the endogenous factors affecting the host’s internal environment. The host’s defence mechanism may be affected by internal systemic factors such as genetic factors, hormones and nutrition (Pack 1988).

Nutrition, especially vitamin C, has received considerable attention in the periodontal researches. Investigations into vitamin C-periodontal relationship go as far back as the 18\(^{th}\) century when a British naval physician revealed that scurvy, which was accompanied by putrid gums could be successfully treated with oranges and lemons (Rubinoff 1989). Since then, numerous experimental as well as epidemiological studies in both humans and animals have been attempted to address this issue.

The effects of vitamin C deficiency in periodontal tissues were first studied by Glickmans (1948a) in guinea-pig. Histological examination revealed the formation of deeper pockets in animals deficient in vitamin C, as well as gingival edema, hemorrhage and increased periodontal destruction (Glickmans 1948b). In monkeys, vitamin C deficiency led to an increased osteoclastic resorption rate and scorbutic gingivitis (Waerhaug 1958, Alvares and Siegel 1981).

Data from studies of patients with acute necrotizing ulcerative gingivitis (ANUG) have provided suggestive evidence for an association between vitamin C deficiency and disease risk. A case-control study of vitamin C plasma level and ANUG showed that patients with a history of ANUG ingested less vitamin C compared to healthy controls. On the other hand, Leggot et al (1986, 1991) found no significant changes in plaque accumulation,
probing depth or attachment level during periods of depletion or supplementation of vitamin C, but the gingival bleeding increased significantly after the period of vitamin C depletion and returned to baseline values after its repletion. In addition, the use of megadoses of vitamin C in normal subjects did not have a predictable or strong effect on the gingival response to initial therapy (Woolfe et al 1984). In contrast, in more recent studies, Nishida et al (2000) showed that a decreased vitamin C intake is a contributing risk factor for periodontal disease, especially in smokers, utilizing data from NHANES III. Sheiman et al (2001) found that the intake of protein, calcium, and vitamin C was significantly lower in edentulous subjects and people with 21 or more teeth consumed more of most intake. Pussinen et al (2003) demonstrated that P. gingivalis infection was associated with low concentration of vitamin C plasma level, which may increase colonization of P. gingivalis or disturb the healing of the infected periodontium. The relationship between plasma level of vitamin C and periodontitis was examined in a community-dwelling elderly in Japan and it is showed that vitamin C plasma level might have relatively weak but statistically significant relationship with periodontitis (Amarasena et al 2005). Furthermore, Staudte et al (2005) conducted a study to examine the vitamin C plasma level and inflammatory measures in periodontitis patients before and after the consumption of grapefruit. It was found that periodontitis patients are characterized by plasma vitamin C levels below the normal range and that grapefruit consumption reduces the sulcus bleeding scores but not the probing depth.

The longitudinal study on periodontal disease which evaluated the initiation and progression of periodontal disease in an Indonesian rural population deprived from regular dental care has been published (van der Velden et al 2006). The results showed that 20%
of the population developed severe periodontitis. Unfortunately, at the start of the study, 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 is to study in this population the relationship between vitamin C, as assessed by plasma vitamin C level, and the severity of periodontitis.

Material and Methods

This was a continuation of a longitudinal study on initiation and progression of periodontal disease in a population carried out in Indonesia in 1987, 1994, and 2002. The study population was obtained from the Malabar/Purbasari tea estate on West Java, and was described in earlier reports (Van der Velden et al 1993, 1996, Timmerman et al. 1998, 2000, 2001, 2002, Van Winkelhoff et al. 1999). Clinical measurements were performed as described in previous reports (Timmerman et al. 1998, 2000, 2001). Plaque (Silness & Löe 1964), Bleeding on Probing (Van der Velden, 1979), Pocket Depth (PD) and Attachment Loss (AL) were recorded at all approximal surfaces from the vestibular aspects. For the present study in 2005, 123 subjects could be retrieved out of the 128 subjects evaluated in 2002.

After identification of each subject, blood samples were taken by the local hospital staff to measure the plasma vitamin C level. Samples were stored 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 hematocrit. The durability for vitamin C in heparin plasma is 5 days at -20°C. At room temperature however this is only up to 2 hours. To
minimize the oxidation of the vitamin C, blood samples were centrifuged within 10 minutes. The plasma obtained was subsequently subjected to the sample 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 reaction vial, 100µl of the reconstituted Precipitation Reagent that contained the Internal Standard was pipetted and 100µl was added of either standard, control or specimen plasma. Vials were vortexed for 10 seconds. The mixtures were incubated for 10 minutes at + 4°C and centrifuged for 5 minutes at 13 000 rpm (Heraeus Biofuge Fresco, Hanau, Germany). The supernatants obtained from these procedures were kept in the refrigerator at 4°C and delivered to the laboratory in Bandung to evaluate plasma vitamin C levels using 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 for the injection volume 20µl, run time about 5 min, flow rate at 1-1.5 ml/min, column temperature approximately 25°C with UV detector’s wavelength of 245 nm (Hewlett Packard HPLC Instrument, HP-1100, Ontario, Canada). To assess the plasma vitamin C levels, 20µl supernatant was injected into the HPLC system. The concentration of vitamin C in the sample was calculated according to the manufactur’s instructions. Plasma vitamin C levels were categorized according to internationally established limits: deficiency (less than 2mg/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 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. 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. Stepwise multiple linear regression analysis was used to test possible association between plasma vitamin C level and attachment loss (AL). $p$-values of <0.05 were accepted as statistically significant. AL was entered as the dependent variable and plasma vitamin C level, age, gender, smoking status and education as independent variable in the stepwise layer of the model.

**Results**

In 2005, 123 subjects out of the 128 could be retrieved and were able to participate in this study. This included 64 females and 59 males, aged of 33-43 year old. Five subjects dropped out because they were not available for assessment at the time of the study.

Plasma levels of vitamin C ranged from 0.02 to 34.45 mg/l with a mean of 7.90 mg/l (SD=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
gender (male versus female: 6.97 (±4.49) and 8.35 (±5.67) mg/L, respectively, p=0.14). Among males, 16.9% were deficient for vitamin C, whereas 12.5% females showed deficiency.

In order to determine the relationship 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 education. The results showed that only vitamin C and education were significantly related to the severity of attachment loss explaining 3.9% and 3.3% of the variance (table 6).

The mean frequencies of monthly food intake based on dietary questionnaire….It can be seen that only rice, onion, chili and garlic were eaten on a daily basis. Mean frequency of monthly food intake by level of vitamin C contents in relation to the plasma vitamin C level category is shown in table 5. Food containing less than 2 mg vitamin C/100 gr was classified as no vitamin C, 2–20 mg vitamin C/100 gr as low, 21–60 mg vitamin C/100 gr as fair, and 61–280 mg/100 gr as high sources of vitamin C. No statistical significant differences could be assessed in frequency of food intake regarding vitamin C between the four plasma vitamin C level categories.

Discussion

The findings of this cross-sectional study suggested that there was a significant association between the level of plasma vitamin C and periodontitis as measured by means AL as assessed in 2002. In other words, it was observed an inverse independent relationship between plasma vitamin C level and AL - the lower the level of plasma vitamin C the higher was the periodontal attachment loss.
The Recommendation Daily Allowance (RDA) for vitamin C intake as stated in Indonesia Nutrition Network is 60 mg for adults. 80% to 90% of vitamin C is absorbed and will maintain an adequate body pool, which is the RDA standard for males and females from ages 15 to 60. The trends of vegetables and fruits intake which are the best sources of vitamin C in this study population were low. As this study population comprised of that an adult population in a remote area, the diet pattern was different compared to that of urban areas. The total energy intake was higher in this rural area, but protein intake was higher in urban areas. Cereal consumption in rural areas was higher than urban areas, and the quality of diets in urban areas was better than in rural areas (Nandi 2000). Compared to dietary intake in industrialized country, the total monthly intake of vegetables and fruits per capita consumption was 28.92 kg in United States, which can be considered high (Frazao 1999). Low intake in vegetables and fruits is caused not only by inadequate food availability but also literacy and education, especially among women (Nandi 2000). It is in accordance with a study by Giskes et al. 2004 conducted in the Netherlands that the least educated group had higher intake of total fat and were less likely to consume fruit and vegetables than the most educated group.

Another factor that may affect the vitamin C intake is how the food sources are prepared. In rural area, the availability of green leafy vegetables and fruits are high, especially in agricultural area, and the diet may contain adequate amounts of fruit and vegetables. However, all the fruit and vegetables undergo prolong cooking, which probably destroy their vitamin C content. The consumption of chili, which was considered to be rich in vitamin C, was high, but it was not consumed in a considerable amounts and did not enter into the diet to any great extent.
In spite of different plasma vitamin C categories among subjects, there were no differences in mean frequency of monthly intake of vitamin C sources by level of contents (table 5). One explanation of these results would be the differences in vitamin C absorptions among subjects. A possible factor that may alter the vitamin C intake is Helicobacter pylori infections. Plasma vitamin C level was inversely related to seroprevalence of H. pylori among whites enrolled in NHANES III (Simon et al. 2003). Vitamin C levels in gastric juice, plasma and whole blood showed a significant negative correlation with the density of Helicobacter pylori on histologic analysis in Korean children (Park et al. 2003). Another study reported that the mean plasma vitamin C concentration in those who were H. pylori-positive was only 65% of that in those classified negative (Woodward et al. 2001). Furthermore, it was demonstrated that H. pylori substantially impaired the bioavailability of dietary vitamin C (Woodward et al. 2001).

Cigarette smoking has been strongly associated with both the prevalence and the severity of periodontitis (Bergström & Preber 1994, Albandar et al. 2000, Bergström et al. 2000a,b). Moreover, cigarette smoking is a factor associated with deeper periodontal pockets and an intra-oral distribution that is suggestive of a local effect (Van der Weijden et al. 2001). Surprisingly, in this present study, there is no significant relationship between smoking and attachment loss. One explanation for the absence of this relationship may be that the smokers in this population commonly smoke kretek cigarettes. Kretets are also known as clove cigarettes, as they typically contain 40% cloves and 60% tobacco. The cloves would serve to provide aroma while also contributing eugenol, a natural component of cloves and a sensory deadening agent to numb the airways while smoking (Fowless
Eugenol belongs to the group of essential oils that possess relatively strong antimicrobial properties (Kalamba & Kunicka 2003) possibly compensating for the negative impact of tobacco smoking.

Previous studies have documented differences in periodontal health by socioeconomic status, i.e. income and education (Locker & Leake 1993, Borrell et al. 2005). In the present study, it is shown that education is related to attachment loss. It is in accordance with a study by Borrell et al. (2006) suggested that compare to those with more than a high school education, those with less than a high school diploma were twice as likely to to have periodontitis. Moreover, it was suggested that education has a greater influence than income in favourably affecting the level of periodontitis in the population (Borrell et al. 2004).

Various researchers have proposed several plausible biological mechanisms while attempting to explain how vitamin C could affect the healthy tissues in humans as well as in animals (Glickman 1948a,b, Rubinoff et al. 1989, Enwonwu 1995, Leggot et al. 1986,1991, Melnick et al. 1988). It has been established that vitamin C plays a major role in the synthesis of collagen which is undoubtedly an essential component of human tissues including periodontium and required in wound healing as well as periodontal regeneration and maintaining the the integrity of the gingival vasculature (Leggot et al. 1986, 1991). Furthermore, some investigators have reported that the deficiency in vitamin C levels could be linked to increased permeability of gingival mucosa, which allows easy passage of microbial and other noxious products into the periodontium. It has also been shown that vitamin C demonstrates antioxidant properties and therefore is considered one of the constituents of antioxidant defence mechanism in human body (Nishida et al. 2000).
In addition, a hypothesis that periodontitis is associated with vitamin C is supported by the investigations that additional vitamin C is required during infectious diseases, due to increased oxidative stress (Enwonwu et al. 2002, Field et al. 2002). It is well established that periodontitis is a chronic infection caused predominantly by gram-negative bacteria, especially A. actinomycetemcomitans and P. gingivalis (Consensus Report of the 1996 World Workshop in Periodontics). Vitamin C is highly concentrated in leucocytes and is used rapidly during infection to prevent oxidative damage. In humans, the essentiality of vitamin C to the immune system is most clearly illustrated during scurvy, where infections occur and where poor responses are measured throughout the whole immune system (Orten et al. 1982).

In conclusion, our study demonstrated that plasma vitamin C level was statistically significantly associated with periodontitis and level of education attainment, particularly high school education. Smoking status, age and gender were not related to attachment loss in this study population. Further studies are needed to determine if vitamin C will improve periodontal health and to investigate the effect of kretek smoking compared to cigarette smoking in relation to attachment loss. Nutrition education and dietetic counseling are required to promote the dietary habits among this study population and in turn might have positive effect in overall health condition.

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