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Antioxidants as Risk Factors for Gingival Bleeding

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CONTENTS

LIST OF TABLES

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ABSTRACT

Background: Studies of gingival bleeding and the effects of antioxidants on extracellular matrix and immunologic and inflammatory responses provide a rationale for hypothesizing that antioxidants reduce the risk for gingival bleeding.

Methods: This study evaluated the role of antioxidants as contributing risk factors for gingival bleeding utilizing the Third National Health and Nutrition Examination Survey (NAHNES III). A sample of 18,825 adults (20 to ≥ 90 years of age), with dental measurement and assessment of serum levels of antioxidants were included in the study. Gingival bleeding was defined as those who had more than 30 percent of gingival bleeding in 28 sites examined. SPSS version 11.0 software and Epi-info 2000 were used to perform the statistical analysis.

Results: Using multiple logistic regression in five separate antioxidants, the study showed an association between increased plasma levels of vitamin C (ascorbic acid) and decreased risk for gingival bleeding (OR= 0.33; 95% CI 0.15 to 0.72). An inverse relationship was also found between gingival bleeding and serum levels of beta carotene (OR=1.93; 95% CI 1.05 to 3.54). However, negative association was found between gingival bleeding and vitamin A (OR=2.60; 95% CI 1.04 to 6.50). No statistically significant association was observed between gingival bleeding and serum levels in vitamin E (alpha tocopherol) and selenium.

Conclusion: Antioxidants, vitamin C, vitamin A, and beta carotene, were significant risk factors for gingival bleeding. This should be emphasized for improving the oral health of the U.S. adult population.

Key words: gingival bleeding, antioxidants, NAHNES III, multiple logistic regressions, vitamin C (ascorbic acid), vitamin E (alpha tocopherol), beta carotene, vitamin A, selenium.

INTRODUCTION

Periodontitis is a disease of tooth-supporting structures resulting from the complex interaction between pathogenic bacteria and the host's immune response[1-3]. Periodontitis is initiated by the colonization of the gingiva by bacterial pathogens, such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* and *Tannerella forsythus*, of which *P. gingivalis* is of most significant[4]. These bacteria have the ability to activate host defense mechanisms which break down the epithelia and other structures of the gum, while at the same time inactivating repair systems[5, 6].

Accumulation of plaque and calculus produces a low-grade inflammatory response in the gums. Once the inflammatory process gets started, reduction in attachment loss occurs, and teeth loosen, eventually would be extruded, and lost due to bony and connective tissue support. Among host's responses, polymorphonuclear leukocytes serve as the initial host defense against these periodontal pathogens. After stimulation by bacterial antigens, polymorphonuclear leukocytes produce singlet oxygen (O_2^-), a reactive oxygen species (ROS), during phagocytosis. Hopochlorous acid (HOCl), another ROS, is produced by myeloperoxidase during phagocytic degranulation[7]. These ROS contribute to tissue destruction by damaging DNA and protein, causing lipid peroxidation and oxidation of other important enzymes (such as antiproteases) and

stimulating proinflammatory cytokine release by monocytes and macrophages[8]. Several studies have demonstrated a correlation between ROS and periodontal disease activity[9-11]. The damages mediated by ROS can be mitigated by antioxidants through three separate mechanisms: scavenging of free radicals as they form, sequestering transition metal ions, and catalyzing oxidation of other molecules[12]. Major antioxidants that impact the immune response include vitamin C (ascorbic acid), vitamin E (α-tocopherol), Vitamin A/β-carotene, and selenium[13].

Vitamin C (ascorbic acid)

Vitamin C (ascorbic acid) is known as one of several compounds that form part of the body's antioxidant defense system. It has been reported that vitamin C directly neutralizes free radicals[15], scavenges the hydroxyl radicals which mediate tissue damage[14], and suppresses macrophage secretion of superoxide anions[16]. Studies of vitamin C and periodontal disease have produced mixed results. Although several studies have not shown a clear relation between plasma ascorbic acid levels and inflammatory periodontitis, an epidemiologic study of vitamin C intake demonstrated a positive association between low dietary vitamin C intake and periodontal disease, especially among smokers[17, 18]. However, mixtures of ascorbic acid with iron or copper

ions can accelerate oxidative damage in vitro: this is often dismissed as irrelevant in vivo because such ions are usually safely protein-bound[19, 20]. However, "free" iron or copper ions can be released at sites of tissue injury[19-21] and there is increasing evidence to link high body-stores of iron or copper to human disease[22]. Thus although vitamin C deficiency should be avoided, megadoses of vitamin C are not to be recommended, especially in sick or old people (who often have high body iron-stores) [23].

Vitamin E (α-tocopherol)

Vitamin E terminates the free radical chain reaction and stabilizes membrane structure, but the molecule has limited mobility, which restricts its efficacy[24]. Like vitamin C, no statistically significant differences have been found in plasma vitamin E between individuals with and without periodontal disease, although these studies of gingival tissue have suggested a mitigating effect of vitamin E on periodontal inflammation and collagen breakdown and lower gingival levels vitamin E among those with periodontal disease compared with healthy controls[25-27].

Vitamin A/β-carotene

Vitamin A/β-carotene function as radical-trapping antioxidants. With the exception of

Papillon-Lefevre syndrome, few researches had been done investigating the role of carotenoids in periodontal disease. Although increasing epidemiological evidence that high intake of such antioxidant is associated with diminished risk of cancer and cardiovascular diseases, especially in smokers[28], retinoids had no observed positive influence on periodontal status[29]. Recent genetic research has reported that defects in polymorphonuclear leukocyte functional enzymes are responsible for the Papillon-Lefevre syndrome[30]. In animal studies, supplemental vitamin A increases the breaking strength of healing wounds, and in vivo it has enhanced collagen accumulation[31].

Selenium

Selenium behaves both as an antioxidant and anti-inflammatory agent. This is because selenium is its antioxidant role, can reduce hydrogen peroxide, lipid and phospholipids hydroperoxides, thereby dampening the propagation of free radicals and reactive oxygen species; reduce hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways diminishing the production of inflammatory prostaglandins and leukotrienes[32]. Recent evidence has reinforced the importance to health of adequate selenium status. Selenium intakes may be suboptimal with respect to disease risk, in the disease of cancer[33, 34] and HIV progression to AIDS[35-38]. Studies also demonstrated

selenium with glutathione peroxidase reduces the radical-derived H_2O_2 content thus preventing the further deterioration by the radicals, which increase the proteolytic activity[39, 40].

Other factors

Several decades age, obesity was noted to contribute to the severity of periodontal disease in rats[41]. Only recently has obesity been noted to be risk factor for periodontal disease in human studies. In a study of Japanese adults, Saito et al. associated increasing body mass index and waist-to-hip ratio with increased risk of periodontitis[42]. In a study of older adults in New England, increased body mass index was associated with gingival bleeding and periodontal disease was associated with weight gain[43]. Obesity, like smoking, may have the potential of modulating the host's immune and inflammatory system, rendering the patient more susceptible to the effects of microbial plaque[12]. Individuals who are obese may ingest larger quantities of nutrient-poor, calorie- and saturated fat-dense foods that may contribute to poor overall oral health.

Smoking is known as a strong risk factor for periodontal disease[44] and is also known to contain numerous oxidants causing tissue damage[45]. There are several reports indicating

that smokers have low blood levels of vitamin C [46, 47] and higher metabolic turnover and decreased efficiency of absorption of vitamin C than non-smokers[48, 49]. Given the potentially modulating role of antioxidants on the detrimental effects of smoking, the interaction between smoking and antioxidant status should be more and carefully addressed.

Nutrition factors in terms of antioxidants may play an important role in gingival bleeding. The aim of the present study is to investigate an association between antioxidant status and periodontal condition defined Generalized Gingival Bleeding (GGB) using the National Institute of Health Periodontal probe.

METHODS

Data Source

The current study utilized the third National Health and Nutrition Examination Survey (NAHNES III) which was collected, analyzed, and disseminated by The National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) on the health status of U.S. residents. The third NAHNES III, conducted from 1988 through 1994, was the seventh in a series of these surveys which were designed to produce nationally representative data.

The sampling design of the surveys followed a complex, stratified, multi-stage, clustered sample on the U.S. civilian, non-institutionalized populations and 39,695 subjects, aged from one to more than ninety years of age. The NHANES III survey consisted of a home interview followed by a detailed medical examination in a mobile examination center (MEC). A home examination option was employed for the subjects who were unable to visit the MEC. The sample used for the current study consisted of persons 20 years and older from populations in both phases of the NHANES III survey. The original data consists of four datasets; adult data, youth data, exam data, and laboratory datasets, containing all of the dietary, demographic, clinical, biochemical,

dental examination and medical histories, and other health information obtained from the NCHS, Hyattsville, Maryland. [50, 51, 52]

Study Populations and Characteristics

Of the 39,695 subjects selected in this study, 86 percent (33,994) were interviewed, and of these, 78 percent (30,818) were examined in the mobile examination center for the detailed medical check-up. Among 30,818 subjects examined, 18,825 individuals who were aged 20 years and older with at least 7 natural teeth in each arch received a standardized dental examination.[53] Demographic information, socioeconomic status, lifestyle and dental status variables were identified and categorized in accordance to existing literature. Demographic information, included age by decade (20-29 years, 30-39, 40-49, 50-59, 60-69, and 70 years and older), gender(male and female), race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Mexican American, and Other races), region (Northeast, Midwest, West and South), insurance coverage, and rural/urban areas(urban area indicated central/fringe counties of metro areas of 1million population or more and all other areas are included into rural area). Marital status was also categorized as married, widowed, divorced/separated and never married.

Socioeconomic status included education categorized as never attend, 1-8 years, 9-11years, 12years, and 13years and over. Household income was categorized into ≥ $20,000 and <$20,000. Poverty income ratio is a calculated variable based on family income and family size using tables published each year by Census Bureau in a series "Current population reports" on poverty in the United States. The variable was analyzed both as a continuous and categorized variable (<1 and ≥ 1). Lifestyle variables included current cigarette smoking, number of cigarettes per day, and history of diabetes. Information on cigarette smoking was determined from the questionnaire. We also estimated pack years by multiplying the number of years each subject smoked by average number of cigarettes per day. Blood levels for cotinine were considered in the analysis. Cotinine was analyzed both as a continuous and categorized variable. It was categorized into <25ng/ml, 26-74ng/ml, and ≥ 75ng/ml. However, cotinine was not included in the statistical analysis since it was collected only during the first phase of the survey form October 1988 to March 1992 which resulted in a significant amount of missing data. Obesity was measured by the body mass index (<25 normal, 25-29 overweight and ≥ 30 obese) and waist-hip ratio (<0.90 normal and ≥ 0.90).

The major independent variables of interest considered as risk factors for periodontal

disease included serum levels of antioxidants: vitamin A, C, E, Beta-carotene, and selenium. To examine the distribution and significance of the antioxidant serum levels, we first analyzed antioxidants as continuous variables using independent t-test. Vitamin C was, analyzed both as continuous and categorical variables, categorized into <22.14mmol/l, 22.14-42.01mmol/l, 42.02-50.04mmol/l, and >50.04mmol/l. Vitamin A was categorized by <40ug/dl, 40-52ug/dl, 53-68ug/dl, and >68ug/dl and also examined as a continuous variable. Vitamin E was evaluated as both continuous and categorical variables and it was classified into <17.95umol, 17.95-22.51umol/l, 22.52-31.17umol/l, and >31.17umol/l. Beta-carotene which is provitamin A was analyzed as a continuous and categorical variable and classified into <9ug/dl, 9-15ug/dl, 16-27ug/dl, and >27ug/dl. Finally, selenium was also categorized as <112ng/ml, 112-121ng/ml, 122-135ng/ml, and >135ng/ml and evaluated both as continuous and categorical variable.

Classification of periodontal disease status

The oral health examination consisted of a visual and tactile oral and dental examination performed by a licensed dentist specially trained in the use of specific epidemiologic indices for oral health. Before beginning the examination the dentist asked a series of questions about the health status of the respondent, including specific questions on heart

problems and conditions that might require antibiotics before the examination. The examinee was excluded from the periodontal components in case of a positive response of any of the above questions. Periodontal assessments including measurement of gingival bleeding, calculus, pocket depth, and clinical attachment level were carried out in randomly assigned half-mouths, one upper quadrant and one lower quadrant selected. All fully erupted teeth in each quadrant were assessed. Assessments were made on 2 sites, buccal and mesial-buccal, per tooth in each quadrant for a maximum of 28 sites. The distance from the cemento-enamel junction (CEJ) to the free gingival margin and the distance from the free gingival margin to the bottom of the pocket were assessed. The clinical mean attachment level (CMAL) was calculated from these 2 measurements [52]. To determine an association with antioxidants and periodontal status, we first looked at the gingival bleeding, the distribution of probing pocket depth, and the mean attachment loss of all examined teeth. The presence or absence of gingival bleeding was examined in a maximum of 14 teeth for each of the buccal and mesial-buccal sites.[54] A score for gingival bleeding was created by adding the number of bleeding sites. Presence of gingival bleeding was categorized into <30% and ≥ 30% defined as localized and generalized bleeding, respectively. The categorized gingival bleeding was used for analysis as a dependent variable in a positive association with probing pocket

depth and CMAL. Both probing pocket depth and CMAL were analyzed as both continuous and categorical variables. We did not include calculus measures as an independent variable, since we found that it is highly correlated with CMAL, probing pocket depth and gingival bleeding. For this study, study subjects who were less than 20 years old and those who do not have complete dental examination were excluded from the study.

Statistical Analysis

To determine the distribution of the study population, mean, standard error, and percentage in both unweighted and weighted values were calculated. This study utilized the unweighted data for analysis. To assess the association between gingival bleeding and the selected independent variables and covariates, bivariate analysis was performed. Statistical analyses for both continuous and categorical variables were carried out for the selected serum antioxidant levels. T-test and p-values were used to determine significance levels for continuous variables and Odds ratio and 95% CI were calculated for categorical variables. From the bivariate analysis, variables that showed a significance of less than 0.25 were chosen for inclusion into the logistic regression model. Multiple logistic regression analysis was used to examine the association

between an individual subject's gingival bleeding and antioxidants (vitamin A, C, E, beta-carotene, and selenium) in serum levels controlling for the effect of confounders. Because of collinearity problem between the antioxidants, separate logistic regression modeling was utilized for each antioxidant. The SPSS version 11.0 software and Epi-info 2000 were used to perform the statistical analysis.

RESULT

Table1. shows the characteristics of the study population. The mean age of the population is 49.5 years old with the SE of 0.15. Females constitute about 53 percent of the study sample. The majority of the study population was non-Hispanic, whites, married and has 12 years or more education. Mean levels of vitamin C, A, E, beta carotene and selenium were 42.8mmol/l, 52.2ug/dl, 23.9umol/l, 18.7ug/dl and 121.9ng/ml respectively. About 11 percent of the population had generalized gingival bleeding and only 6.4 percent of the study population had clinical mean attachment loss (CMAL) of $\geq$1.5 mm.

Table.2 described the prevalence of generalized gingival bleeding (GGB) of the study population. The prevalence of GGB is evenly distributed across the different age groups. Slightly lower prevalence (8.4% 95%CI 6.24, 11.21) is observed among 60-69 years old. The prevalence of GGB is also evenly distributed among the different genders, racial/ethnic groups, education and income levels, and regions. Higher prevalence was shown among widowed women (13.3 % 95%CI 10.48, 16.83). No major difference was also observed in the prevalence of GGB among the different quartiles of the antioxidants.

The prevalence of GGB among diabetics was slightly higher (12 percent) than non-diabetics. In the measure of body mass index, we had 51 percent of GGB subjects in <25 group, followed by 29 percent in 25-74 and 20 percent in ≥ 75 group. The prevalence of GGB was increased with an increase of BMI. The same trend of the prevalence was observed as BMI was categorized into <24, 25-29, and ≥ 30, although more subjects of GGB in <0.90 were observed than ≥ 0.90 waist to hip ratio group. There was a higher prevalence in ≥ 0.90 group with 14 percent, 95%CI [12.27, 16.16].

Current smokers consisted of 52 percent in GGB and non smokers were 48 percent. The chance of getting GGB was almost same in both groups with 10 percent. The prevalence of GGB among smokers was higher in both ≤ 10 and 11-20 groups as compared to >20 cigarettes group. More than 60 percent of the population with cotinine levels <25 reported having had a GGB, followed by 35 percent in ≥ 75 cotinine levels. The least was observed in 25-74 level of cotinine (2.1 percent). The prevalence was the highest in ≥ 75 (14 percent) level of cotinine. There was higher prevalence of GGB in triglyceride levels of 151-200mg/dl than any other groups. More than 80 percent of the total GGB population had CMAL <1.5mm and 99.2 percent had pocket depths of <2mm.

The unadjusted Odds ratios and 95%CI are displayed in table 3. As compared to

married people, widowed people were almost 3 times more likely to develop greater risk of developing GGB with Odds ratio=0.34 and 95%CI [0.99, 1.80]. A statistically significant association was found between gingival bleeding and waist hip ratio and BMI. People with waist to hip ratio of ≥ 0.90 were a 1.6 times more at risk of gingival bleeding than people with <0.90. Obese (BMI ≥ 30) were 1.3 times more likely to have gingival bleeding as compared to people with < 24 BMI. Subjects with a household income of <$20,000 had a decreased risk of GGB with an Odds ratio of 0.84 and 95%CI [0.68, 1.04]. Among the antioxidant factors (vitamin C, A, E, beta carotene, and selenium), we found that subjects with GGB had a significant association in all five antioxidant serum levels when the analysis was done as continuous variables. However, the crude analysis showed no statistically significant association between gingival bleeding and the selected antioxidants in quartiles. The risk of developing GGB increased with a triglyceride levels of 151-200mg/dl (OR=1.33, 95%CI [0.95, 1.88]) and LDL levels of 101-190mg/dl (OR=1.12, 95%CI [0.70, 1.77]). There was also statistically significant association between gingival bleeding and clinical mean attachment loss (OR=2.02, 95% CI [1.42, 2.89]) and probing pocket depth (OR= 8.81, 95%CI [1.77, 43.83]).

The adjusted Odds ratio is displayed in table 4. When adjusted for race/ethnicity, marital status, waist to hip ratio, family income, urban/rural area, triglycerides, and LDL generalized gingival bleeding was found to be significantly associated with vitamin C, vitamin A, and beta carotene. As compared to subjects with serum vitamin C levels of 22.14mmol/l, subjects that have serum vitamin C level of >50.04mmol/l have a three fold decreased risk of gingival bleeding (OR=0.33, 95%CI [0.15, 0.72]) (Model 1). Model 2 illustrates the association between vitamin A and gingival bleeding after controlling for other confounding factors. As compared to serum vitamin A levels of <40ug/dl, people with serum vitamin A levels of >68ug/dl have 2.6 times higher risk for gingival bleeding (95%CI [1.04, 6.50]). Although not statistically significant, a dose-response was shown between gingival bleeding and vitamin A. The adjusted analysis demonstrated a two fold increased risk for gingival bleeding among people with serum beta carotene levels of >16ug/dl (95%CI [1.05, 3.54]) as compared to people with serum beta carotene levels of <9ug/dl. No statistically significant associations were found between vitamin E or selenium and generalized gingival bleeding in the adjusted logistic regression. However, a statistically significant association was observed when the variables were analyzed as continuous variables. Although not significant, overall increased risk was found in serum vitamin E and protective effect of serum selenium for

gingival bleeding. Waist to hip ratio remained as a significant risk factor with adjusted Odds ratios increasing when compared to the crude Odds ratios. Compare to subjects living in the metro areas, people living in other than metro areas had a decreased risk for gingival bleeding.

DISCUSSION

In this study, we found a statistically significant association between poor oral health in terms of generalized gingival bleeding (GGB) and serum levels of antioxidants appeared to be in only ascorbic acid, beta-carotene, and vitamin A. Based on the adjusted Odds ratios we found that antioxidants were significant factor for GGB. The association remained significant on controlling for GGB (Generalized Gingival Bleeding) factors such as waist to hip ratio and urban/rural area. Our findings were consistent with other studies, which showed an inverse association between gingival bleeding and above-mentioned serum levels of antioxidants particularly in ascorbic acid and beta-carotene. However, we have found that higher serum level on vitamin A had a negative association with the outcome of the disease.

We have found the consistency of our study on ascorbic acid in serum level with several reports from other studies[17,18,55] and the protective effect of OR=0.33 between vitamin C and generalized gingival bleeding (≥ 30%) determined that vitamin c as a major antioxidant is highly related to gingival bleeding.

Leggot et al.[17] (1991) described the relationship between varying vitamin c intakes, periodontal status, and subgingival microflora. No significant changes in plaque

accumulation, probing pocket depth, or attachment level were noted when different vitamin C groups were compared. In contrast, gingival bleeding increased significantly after the period of vitamin C depletion and returned to baseline values after the period of vitamin C repletion. However, no relationship could be demonstrated between either the presence or proportion of target periodontal microorganism and measures of bleeding or vitamin C levels.

A recent cross-sectional report by Nishida et al.[18] (2000 a, b) was able to evaluate the role of dietary vitamin C as a contributing risk factor for periodontal disease utilizing the third National Health and Nutrition Examination Survey (NAHNES III). A sample of 12,419 adults with dental measurements and assessment of dietary information as well as demographic and medical histories, were included in the studies. Using multiple logistic regression analysis, a relationship between reduced dietary vitamin C intake and increased risk for periodontal disease for the overall population was found. There was also a dose-response relationship found between the levels of dietary vitamin C and periodontal disease. The authors concluded that dietary intake of vitamin C showed a weak, but statistically significant, relationship to increased susceptibility for the development of periodontal disease.

More recently Nowjack-Raymer et al.[55] (2002) assessed the National Diet and Nutrition

Survey to determine if there is a relationship between dental status in the US adult population and intake of certain nutrients. Biochemical analyte levels for nutrients found in fruits and vegetables were statistically significantly lower among denture-wearers. The serum beta carotene level among denture-wearers was 1.7 times lower than that of the fully dentate, 1.3 times lower for serum folate, and 1.1 times lower for serum vitamin C than for that of the fully dentate.

In our analysis, we also found significance between serum carotenoids level and GGB in the study population. The magnitude of association of OR was 2.60 (95%CI 1.04, 6.50) for vitamin A and OR=1.93 (95%CI 1.05, 3.54) for beta carotene respectively in terms of the association with GGB. This suggested that beta carotene may also have an independent effect on immune responses, separate from its provitamin A activity. Beta carotene and other carotenoids (which may or may not have provitamin A activity) with nine or more conjugated double bonds may enhance immune function by quenching singlet oxygen and other reactive oxygen species, including free radicals vitamin A, in contrast, cannot quench singlet oxygen and is a relatively poor antioxidant.[56, 57] Since vitamin A is a relatively poor antioxidant and cannot quench singlet oxygen. Beta carotene may have more importance as a nutrient than simply serving as a precursor of

vitamin A.

Sahyoun et al.[58] (2003) analyzed the role of nutrition of the older adult associated with dentition status. The study used data from 5,958 participants in NAHNES III ages 50 years and over with dental examination. In this study, they found a positive association between number of teeth, particularly pairs of occlusal posterior teeth, and the nutritional status of individuals as determined by dietary quality and serum levels of nutrients, and an inverse association between individuals with one to four posterior pairs of teeth and BMI. The variations in food intake patterns by dental status were reflected both in lower intakes of specific nutrients (vitamin A, carotene, vitamin C, folate, and fiber) as well as in lower blood levels of carotene and vitamin C, and in folate in subjects with full dentures. Serum vitamin C and beta carotene levels were significantly and positively associated with number of pairs of posterior teeth. These associations were consistently found for beta carotene when the other dental variables were used in the model. Serum vitamin C level was also significantly lower among participants with fewer than 18 teeth compared with those with more than 18 teeth, but not when the dental variables were examined as continuous. No association was found with vitamin E.

Although there are several studies have suggested mitigating effect of vitamin E on periodontal inflammation and collagen breakdown we have not found any significant

association between vitamin E or selenium and gingival bleeding in our study.

Slade et al.[59] (1976) investigated a relationship between serum vitamin E and periodontal disease. Twenty four subjects participated in the study 12 with inflammatory periodontal disease and 12 without. The results reported that alpha tocopherol per 100ml of plasma showed no significant correlation with inflammatory periodontal disease. Serum vitamin E levels may be a misleading variable for assessing the vitamin E status of the tissues. Horwitt et al.[60] stated that there is a tendency for serum alpha tocopherol to rise and fall in proportion to the cholesterol, phospholipids and triglycerides present in the serum. They suggested that serum tocopherol is related to serum total lipids and should be reported as such.

A recent study conducted by Asman et al.[27] (1994) showed the importance of antioxidants in inflammatory tissues. Degradation of homologous H-collagen powder by experimental granulation tissue induced by cellulose sponges in the rat was monitored as the radioactivity excreted in urine. By administering pharmacological doses of both vitamin E and selenium subcutaneously and by injection into sponges implanted subcutaneously, this breakdown of collagen was reduced. Injections in the sponges also arrested the maturation of the granulation tissue. They concluded that the antioxidants, vitamin E and selenium, reduced collagen degradation in experimental

granulation tissue since vitamin E and selenium are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells. Therefore, they suggested that above-mentioned agents that can reduce the protein degradation in granulation tissue are of importance in locally destructive inflammatory disease, as in periodontitis.

With respect to marital status, our finding is a one and a half fold increased risk of GGB in widowed population with all five antioxidants when analyzed with antioxidants treated as continuous variables. However, we did not found any significance in categorical antioxidants. Waist to hip ratio was found to be significant in both crude odds ratios and adjusted odds ratios. The magnitude of association of POR=1.61 (95%CI 1.31, 1.99) in crude odds increased up to POR=2.08 (95%CI 1.26, 3.42) in vitamin C model as well as increased in the other four models. This founding was consistent with other studies.[61, 62]

Perlstein M.I. et al.[61] studied Influence of obesity and hypertension on the severity of periodontitis in rats. They evaluated the extent to which obesity and/or hypertension may modify the response of rats' periodontium to chronic gingival irritation. Forty-four normal, spontaneously hypertensive, obese, and obese-hypertensive rats were used. Histopathologic evaluation of the periodontal structure showed both hyperplasia and

hypertrophy of the walls of blood vessels supplying the periodontium in the hypertensive and obese-hypertensive animals. The results also indicated that obesity significantly contributed to e severity of periodontal disease. Hypertension alone was not a significant factor. The obese-hypertensive rats showed the most severe periodontal response to local irritation.

Saito T. et al.[62] analyzed Relationship between upper body obesity and periodontitis. This study was conducted to clarify the relationship between upper body obesity and periodontitis, since upper body obesity, related to visceral fat accumulation, is known to increase the risk of various adult diseases, especially type 2 diabetes and cardiovascular disease. They examined 643 apparently healthy, dentulous Japanese adults who attended programs at Fukuoka Health Promotion Center. Waist-hip ratio, body-mass index (BMI), and body fat were significant risk indicators for periodontitis after adjustment for known risk factors ($p < 0.002$).

Subjects were divided into four BMI (or body fat) categories. In only the subjects with high waist-hip ratio, higher categories of BMI (or body fat) significantly increased the adjusted risk of periodontitis, compared with subjects with low waist-hip ratios and the lowest category of BMI (or body fat).

The size and design of this NHANES III study allowed us for the adjustment for social and behavioral factors such as smoking status, which has not been possible in other studies. The subjects had the unique advantage of getting an examination done in the household if they could not be examined at the mobile examination center. We also used the information of the periodontal condition measured by the National Institute of Health (NIH) periodontal probe when compared to studies that used a questionnaire to estimate periodontal disease of the population. We measured every subject who had teeth examined in 28 sites of 14 teeth and determined as a generalized bleeding with more than 30 percent of bleeding of the 28 sites and as a localized bleeding with less than 30 percent. We did not include any subjects who were not examined in 28 sites of 14 teeth to observe more accurate association although we lost number of subjects. Our study utilized data from biochemical analysis levels to provide a better picture of nutrient-laden in the body which may not approach recall bias while nutrition epidemiology methods such as twenty-four hour dietary recall and food frequency questionnaire may lead.

Several limitations in this study should be noted. The current study was a cross-sectional design and we could not evaluate antioxidants from dietary intakes, supplements, or interaction of other nutrients. We did not find any significant

association of smoking status interaction with antioxidants and GGB when compared with other studies that have proven. This may be due to the statistical software (SPSS) that we used in comparison to other software programs.

Although relatively little attention has been given by periodontal research, the medical literature has studied the effects of nutritional supplementation among patients receiving various treatment modalities, and at present nutrition is considered to play an important role in wound healing processes. The effects of nutrition on periodontal disease status and response to treatment have been studied using different methods and study models. Several studies have proven various degrees of association between nutritional elements/supplements and periodontal status, and others have reported possible positive influences of nutritional supplementation on periodontal therapeutic outcomes. Most of the results are mainly based on cross-sectional, case-control, and animal diets. Randomized, controlled, longitudinal trials are needed to categorize oxidants as a "risk factor" for periodontal disease. Furthermore, since periodontal disease is dependent on host susceptibility, prophylactic nutrient supplementation for the prevention of periodontal disease onset and progression is still not indicated. Prospective clinical trials comparing treated patients with an optimal nutrition to patients with an optimal nutrition to patients without the same care are needed to provide scientific evidence for

nutritional supplementation among periodontal patients. In addition, future studies should also evaluate how specific nutritional supplements may influence treatment outcomes. Considering relatively healthy population in this study, we might not see significant association what we expected in vitamin E and selenium. Therefore, controlled clinical trials would be suggested to demonstrate response to the causality and producing more predictable outcomes. In conclusion, our results support those from a few other studies that have proven significance. Although gingival bleeding may not be nutritional deficiency, lack of antioxidants is likely to play a role in both predisposing the host to the progression of preexisting periodontal lesions and influence the outcome of periodontal treatment.

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Table1. Demographic Characteristics of the study population

Variables	N	%	Mean/SE
Age	18825		49.5/0.15
20-29	3783	20.1	
30-39	3594	19.1	
40-49	2794	14.8	
50-59	2058	10.9	
60-69	2608	13.9	
≥ 70	3988	21.2	
Sex	18825		
male	8816	46.8	
female	10009	53.2	
Race/Ethnicity	18825		
Non-Hispanic white	8146	43.3	
Non-Hispanic black	5071	26.9	
Mexican American	4893	26	
Other	715	3.8	
Region	18825		
Northeast	2779	14.8	
Midwest	3614	19.2	
South	8018	42.6	
West	4414	23.4	
Marital Status	18730		
Married	11248	60.1	
Widowed	2351	12.6	
Divorced/Separated	2070	11.1	
Never married	3061	16.3	
Education	18825		
Never attended	512	2.7	
1-8 years	4154	22.1	
9-11 years	3098	16.5	
12 years	5591	29.7	
13 or more	5470	29.1	
Income	18350		
< $20,000	9103	49.6	
≥ $20,000	9247	50.4	
Poverty/income ratio	16794		2.42/0.01
< 1	3834	22.8	
≥ 1	12960	77.2	
Urban/Rural area	18825		
Metro areas	9370	49.8	
other areas	9455	50.2	
Dental Examination	17325		
Yes	16824	97.1	
Dentate status	16067		
Teeth present	14180	88.3	
Dental care plan	6368		
yes	3489	54.8	
Mean attachment loss	3586		1.11/0.003
< 1.5mm	3357	93.6	
≥ 1.5mm	229	6.4	
Probing Pocket depth	3586		1.14/0.004
< 2.0mm	3580	99.8	
≥ 2.0mm	6	0.2	
Gingival bleeding	3758		
< 30%	3347	89.1	
≥ 30%	411	10.9	

Table1. Demographic Characteristics of the study population (cont.)

Variables	N	%	Mean/SE
History of diabetes	18800		
yes	1608	8.6	
no	17192	91.4	
Body mass index	16829		23.52/0.05
< 24	10244	60.9	
25-29	3857	22.9	
≥ 30	2728	16.2	
Waist to hip ratio	15717		0.91/0.001
< 0.90	7620	48.5	
≥ 0.90	8097	51.5	
Cigarette smoking	9552		
yes	4799	50.2	
no	4753	49.8	
Cigarettes per day	4703		16.08/0.18
≤ 10	1499	31.9	
11-20 cig	1232	26.2	
> 20	1972	41.9	
Cotinine	11896		56.45/1.27
< 25	7706	64.8	
25-74	324	2.7	
≥ 75	3866	32.5	
Triglycerides	14895		130.0/0.86
< 151	11021	74	
151-200	1817	12.2	
201-500	1886	12.7	
> 500	171	1.1	
LDL	5016		124.47/0.56
< 101	1430	28.5	
101-190	3302	65.8	
> 190	284	5.7	
Vitamin C	13103		42.79/0.23
< 22.14	3191	24.4	
22.14-42.01	3290	25.1	
42.02-50.04	3339	25.5	
> 50.04	3283	25.1	
Vitamin A	14687		52.17/0.15
< 40	3801	25.9	
40-52	4386	29.9	
53-68	4100	27.9	
> 68	2400	16.3	
β-Carotene	14687		18.69/0.16
< 9	3480	23.7	
9--15	4645	31.6	
16--27	4105	27.9	
> 27	2457	16.7	
Vitamin E	14687		23.92/0.09
< 17.95	4103	27.9	
17.95-22.51	4114	28	
22.52-31.17	4113	28	
> 31.17	2357	16	
Selenium	11616		121.85/0.16
< 112	3008	25.9	
112-121	3120	26.9	
122-135	3414	29.4	
> 135	2074	17.9	

Table2. Prevalence and 95%CI of gingival bleeding in the study population

Variable	Total N	Gingival Bleeding G*(%)	t-test	p-value	95% CI
Age	3758				
20-29	764	11.9			[9.74 14.47]
30-39	742	11.9			[9.67 14.46]
40-49	553	10.3			[7.96 13.22]
50-59	411	10.2			[7.54 13.66]
60-69	523	8.4			[6.24 11.21]
≥ 70	765	11.6			[9.49 14.17]
Sex	3758				
male	1761	10.7			[9.34 12.29]
female	1997	11.1			[9.79 12.60]
Race/Ethnicity	3758				
Non-Hispanic white	1603	11.4			[9.92 13.10]
Non-Hispanic black	1022	10.0			[8.25 12.02]
Mexican American	980	10.6			[8.79 12.75]
Other	153	14.4			[9.42 21.18]
Region	3758				
Northeast	565	11.0			[8.53 13.92]
Midwest	741	11.1			[8.94 13.60]
South	1615	10.8			[9.38 12.48]
West	837	11.0			[8.99 13.36]
Marital Status	3737				
Married	2258	10.3			[9.11 11.67]
Widowed	472	13.3			[10.48 16.83]
Divorced/Separated	380	10.5			[7.71 14.16]
Never married	672	11.5			[9.15 14.30]
Education	3758				
Never attended	113	10.6			[5.85 18.17]
1-8 years	811	10.0			[8.05 12.31]
9-11 years	660	9.8			[7.73 12.44]
12 years	1078	10.9			[9.18 13.00]
13 or more	1096	12.3			[10.46 14.44]
Income	3667				
< $20,000	1831	10.0			[8.68 11.48]
≥ $20,000	1836	11.7			[10.24 13.23]
Poverty/income ratio	3361		-1.515	0.13	
< 1	781	10.6			[8.60 13.05]
≥ 1	2580	11.0			[9.80 12.25]
Urban/Rural area	3758				
Metro areas	1887	11.8			[10.36 13.32]
other areas	1871	10.1			[8.79 11.58]

G* Generalized gingival bleeding

Table2. Prevalence and 95%CI of gingival bleeding in the study population (cont.)

Variable	Total N	Gingival Bleeding G*(%)	t-test	p-value	95% CI
Hist. of diabetes	3752				
yes	310	12.3			[8.92 16.56]
no	3442	10.8			[9.83 11.93]
Body mass index	3758		-2.469	0.014	
< 24	2062	10.1			[8.88 11.54]
25-29	1042	11.3			[9.50 13.44]
≥ 30	654	12.8			[10.43 15.71]
Waist to hip ratio	3651		-6.485	<0.01	
< 0.90	2368	9.2			[8.13 10.50]
≥ 0.90	1283	14.1			[12.27 16.16]
Cig. smoking	1904				
yes	1005	10.0			[8.29 12.12]
no	899	10.2			[8.37 12.45]
Cig. per day	985		1.709	0.088	
≤ 10	292	10.6			[7.44 14.87]
11--20	252	11.5			[7.97 16.26]
> 20	441	8.8			[6.44 11.99]
Cotinine	2290		1.428	0.153	
< 25	1488	12.0			[10.38 13.75]
25-74	66	9.1			[3.75 19.39]
≥ 75	736	13.7			[11.36 16.47]
Triglycerides	3000		0.651	0.515	
< 151	2234	10.3			[9.12 11.70]
151-200	338	13.3			[9.97 17.51]
201-500	389	9.3			[7.31 13.56]
> 500	39	2.6			[0.13 15.08]
LDL	983		-0.943	0.346	
< 101	285	9.8			[6.74 14.03]
101-190	645	10.9			[8.61 13.57]
> 190	53	7.5			[2.45 19.07]
Vitamin C	2638		0.744	0.457	
< 22.14	624	11.5			[9.19 14.37]
22.14-42.01	691	9.3			[7.26 11.73]
42.02-50.04	632	10.8			[8.51 13.50]
> 50.04	691	10.1			[8.03 12.68]
Vitamin A	2962		-0.778	0.437	
< 40	756	10.8			[8.76 13.34]
40-52	891	9.4			[7.63 11.59]
53-68	823	10.8			[8.82 13.19]
> 68	492	10.6			[8.06 13.71]

G* Generalized gingival bleeding

Table 2. Prevalence and 95%CI of gingival bleeding in the study population (cont.)

Variable	Total N	Gingival Bleeding G*(%)	t-test	p-value	95% CI	
B-Carotene	2962		0.26	0.795		
< 9	730	9.6			[7.60	12.02]
9--15	925	11.0			[9.12	13.27]
16--27	809	9.4			[7.52	11.67]
> 27	498	11.8			[9.21	15.09]
Vitamin E	2962		-0.32	0.749		
< 17.95	817	9.4			[7.55	11.69]
17.95-22.51	826	10.3			[8.34	12.62]
22.52-31.17	835	11.4			[9.34	13.77]
> 31.17	484	10.3			[7.83	13.48]
Selenium	2345		0.692	0.489		
< 112	615	11.4			[9.04	14.22]
112-121	624	8.7			[6.62	11.21]
122-135	681	9.8			[7.76	12.39]
> 135	425	10.4			[7.70	13.74]
Dental Exam	3758					
Yes	3758	10.9			[9.97	11.99]
Dentate status	3758					
Teeth present	3758	10.9			[9.97	11.99]
Dental care plan	1319					
yes	688	10.3			[8.20	12.90]
no	631	8.9			[6.83	11.44]
Mean attach. loss	3586		-3.95	<0.001		
< 1.5mm	3357	9.7			[8.76	10.80]
≥ 1.5mm	229	17.9			[13.29	23.62]
Pocket depth	3586		-3.215	0.001		
< 2.0mm	3580	10.2			[9.23	11.24]
≥ 2.0mm	6	50.0			[13.95	86.05]

G* Generalized gingival bleeding

Table3. Unadjusted Odds ratios of gingival bleeding in the study population

Variable	Total (N)	GGB* (%)	Crude Odds Ratio	95%CI
Age	3758			
20-29	764	11.9		
30-39	742	11.9	1	[0.73 1.36]
40-49	553	10.3	0.85	[0.60 1.21]
50-59	411	10.2	0.84	[0.57 1.24]
60-69	523	8.4	0.68	[0.47 0.99]
≥ 70	765	11.6	0.97	0.71 1.33]
Sex	3758			
male	1761	10.7		
female	1997	11.1	1.04	[0.85 1.28]
Race/Ethnicity	3758			
Non-Hispanic white	1603	11.4		
Non-Hispanic black	1022	10	0.86	[0.67 1.11]
Mexican American	980	10.6	0.92	[0.71 1.19]
Other	153	14.4	1.3	[0.81 2.10]
Region	3758			
Northeast	565	11		
Midwest	741	11.1	1.01	[0.71 1.43]
South	1615	10.8	0.99	[0.73 1.34]
West	837	11	1	0.71 1.41]
Marital Status	3737			
Married	2258	10.3		
Widowed	472	13.3	1.34	[0.99 1.80]
Divorced/Separated	380	10.5	1.02	[0.72 1.46]
Never married	672	11.5	1.13	[0.85 1.50]
Education	3758			
Never attended	113	10.6	0.85	[0.45 1.58]
1-8 years	811	10	0.79	[0.59 1.06]
9-11 years	660	9.8	0.78	[0.60 1.06]
12 years	1078	10.9	0.88	[0.67 1.14]
13 or more	1096	12.3		
Income	3667			
< $20,000	1831	10	0.84	[0.68 1.04]
≥ $20,000	1836	11.7		
Poverty/income ratio	3361			
< 1	781	10.6	0.97	[0.75 1.25]
≥ 1	2580	11		
Urban/Rural area	3758			
Metro areas	1887	11.8		
other areas	1871	10.1	0.84	[0.69 1.04]
Dental Examination	3758			
Yes	3758	10.9		
Dentate status	3758			
Teeth present	3758	10.9		
Dental care plan	1319			
yes	688	10.3		
no	631	8.9	0.85	[0.59 1.22]
Mean attach. loss	3578			
< 1.5mm	3349	9.7		
≥ 1.5mm	229	17.9	2.02	[1.42 2.89]
Pocket depth	3578			
< 2.0mm	3572	10.2		
≥ 2.0mm	6	50	8.81	[1.77 43.83]

GGB*: Generalized Gingival Bleeding

Table3. Unadjusted Odds ratios of gingival bleeding in the study population (cont.)

Variable	Total (N)	GGB (%)	Crude Odds Ratio	95%CI
History of diabetes	3752			
yes	310	12.3	1.15	[0.81 1.64]
Body mass index	3758			
< 24	2062	10.1		
25-29	1042	11.3	1.13	[0.89 1.44]
≥ 30	654	12.8	1.31	[1.00 1.71]
Waist to hip ratio	3651			
≥ 0.90	1283	14.1	1.61	[1.31 1.99]
Cigarette smoking	1904			
yes	1005	10	0.98	[0.73 1.32]
Cigarettes per day	985			
≤ 10	292	10.6		
11--20	252	11.5	1.1	[0.64 1.87]
> 20	441	8.8	0.82	[0.50 1.34]
Cotinine	2290			
<25	1488	12		
25-74	66	9.1	0.74	[0.31 1.73]
≥ 75	736	13.7	1.17	[0.90 1.52]
Triglycerides	3000			
< 151	2234	10.3		
151-200	338	13.3	1.33	[0.95 1.88]
201-500	389	9.3	0.88	[0.61 1.28]
> 500	39	2.6	0.23	[0.03 1.67]
LDL	983			
< 101	285	9.8		
101-190	645	10.9	1.12	[0.70 1.77]
> 190	53	7.5	0.75	[0.25 2.23]
Vitamin C	2638			
< 22.14	624	11.5		
22.14-42.01	691	9.3	0.78	[0.55 1.12]
42.02-50.04	632	10.8	0.92	[0.65 1.31]
> 50.04	691	10.1	0.86	[0.61 1.22]
Vitamin A	2962			
< 40	756	10.8		
40-52	891	9.4	0.86	[0.62 1.18]
53-68	823	10.8	1	[0.73 1.40]
> 68	492	10.6	0.97	[0.67 1.40]
β-Carotene	2962			
< 9	730	9.6		
9--15	925	11	1.17	[0.85 1.61]
16--27	809	9.4	0.98	[0.70 1.38]
> 27	498	11.8	1.27	[0.88 1.83]
Vitamin E	2962			
< 17.95	817	9.4		
17.95-22.51	826	10.3	1.1	[0.80 1.53]
22.52-31.17	835	11.4	1.23	[0.90 1.70]
> 31.17	484	10.3	1.11	[0.76 1.61]
Selenium	2345			
< 112	615	11.4		
112-121	624	8.7	0.74	[0.51 1.07]
122-135	681	9.8	0.85	[0.60 1.21]
> 135	425	10.4	0.9	[0.60 1.34]

GGB*: Generalized Gingival Bleeding

Table4. Adjusted Odds ratios of gingival bleeding and antioxidants

Characteristics		Model 1 Vit. C OR (95%CI)	Model 2 Vit. A OR (95%CI)	Model 3 Vit. E OR (95%CI)	Model 4 β-carotene OR (95%CI)	Model 5 Selenium OR (95%CI)
Race/Ethnicity						
Non-Hispanic white		1.0	1.0	1.0	1.0	1.0
Non-Hispanic black		0.68 (0.39, 1.21)	0.67 (0.38, 1.18)	0.66 (0.38, 1.17)	0.66 (0.37, 1.16)	0.80 (0.55, 1.15)
Mexican American		0.65 (0.36, 1.17)	0.68 (0.38, 1.24)	0.67 (0.37, 1.21)	0.66 (0.37, 1.20)	0.87 (0.60, 1.25)
Other		0.94 (0.30, 2.94)	0.96 (0.31, 2.97)	0.88 (0.29, 2.70)	0.86 (0.28, 2.63)	1.14 (0.56, 2.32)
Marital Status						
Married		1.0	1.0	1.0	1.0	1.0
Widowed		1.59 (0.78, 3.23)	1.50 (0.75, 2.97)	1.50 (0.76, 2.99)	1.57 (0.79, 3.11)	1.44 (0.94, 2.21)
Divorced/Separated		1.77 (0.86, 3.62)	1.77 (0.87, 3.63)	1.75 (0.86, 3.57)	1.67 (0.82, 3.42)	1.22 (0.76, 1.96)
Never married		1.39 (0.73, 2.65)	1.22 (0.64, 2.33)	1.27 (0.67, 2.41)	1.32 (0.70, 2.49)	1.22 (0.83, 1.81)
W-hip ratio	< 0.90	1.0	1.0	1.0	1.0	1.0
	≥ 0.90	2.08 (1.26, 3.42)	2.03 (1.24, 3.32)	2.03 (1.24, 3.31)	2.02 (1.24, 3.29)	1.73 (1.26, 2.37)
Income	< $20,000	1.0	1.0	1.0	1.0	1.0
	≥ $20,000	1.10 (0.67, 1.78)	1.06 (0.65, 1.73)	1.06 (0.65, 1.72)	1.03 (0.64, 1.67)	1.05 (0.78, 1.43)
Area	Metro areas	1.0	1.0	1.0	1.0	1.0
	other areas	0.55 (0.34, 0.88)	0.61 (0.38, 0.97)	0.60 (0.37, 0.95)	0.59 (0.37, 0.94)	0.73 (0.55, 0.98)
Triglyc.	< 151	1.0	1.0	1.0	1.0	-
	151-200	1.67 (0.90, 3.09)	1.32 (0.70, 2.48)	1.44 (0.77, 2.71)	1.52 (0.82, 2.82)	-
	201-500	1.10 (0.56, 2.18)	0.91 (0.46, 1.80)	0.97 (0.47, 2.00)	1.12 (0.57, 2.20)	-
LDL	< 101	1.0	1.0	1.0	1.0	-
	101-190	1.24 (0.74, 2.07)	1.09 (0.65, 1.83)	1.05 (0.60, 1.85)	1.15 (0.68, 1.92)	-
	> 190	0.67 (0.19, 2.38)	0.57 (0.16, 2.01)	0.55 (0.15, 2.07)	0.60 (0.17, 2.15)	-
Vit.C	< 22.14	1.0	-	-	-	-
	22.14-42.01	0.82 (0.46, 1.46)	-	-	-	-
	42.02-50.04	1.17 (0.65, 2.09)	-	-	-	-
	> 50.04	0.33 (0.15, 0.72)	-	-	-	-
Vit. A	< 40	-	1.0	-	-	-
	40-52	-	1.40 (0.58, 3.40)	-	-	-
	53-68	-	1.78 (0.75, 4.25)	-	-	-
	> 68	-	2.60 (1.04, 6.50)	-	-	-
β-Carotene	< 9	-	-	-	1.0	-
	9--15	-	-	-	1.93 (1.05, 3.54)	-
	16--27	-	-	-	1.22 (0.62, 2.42)	-
	> 27	-	-	-	1.56 (0.78, 3.13)	-
Vit. E	< 17.95	-	-	1.0	-	-
	17.95-22.51	-	-	1.08 (0.58, 2.39)	-	-
	22.52-31.17	-	-	1.42 (0.69, 2.91)	-	-
	> 31.17	-	-	1.38 (0.59, 3.28)	-	-
Selenium	< 112	-	-	-	-	1.0
	112-121	-	-	-	-	0.80 (0.54, 1.17)
	122-135	-	-	-	-	0.92 (0.64, 1.34)
	> 135	-	-	-	-	0.92 (0.61, 1.41)