Neutrophil Function and Periodontitis in Alcohol-dependent Males without Medical Disorders

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Abstract

Background: Periodontitis and immune dysfunction are often reported in alcohol-dependent patients. Our objectives were to investigate the effects of alcohol exposure on neutrophil function and the associated consequential effects on the periodontium in a group of African American (AA) males with documented history of alcohol use without medical complications. **Methods:** Thirty-three AA males with documented history of alcohol use were included in this analysis. All subjects were free from systemic illness. Blood levels of gamma-glutamyl transpeptidase (GGTP) were determined and used as a measure of alcohol consumption. Periodontal evaluations including attachment levels (AL) were recorded on 6 sites per tooth. Enumerative and functional neutrophil measures were obtained. **Results:** GGTP blood levels inversely associated with neutrophil bacterial killing (NBK) ($p = 0.04$). Regression analysis, adjusting for risk factors associated with periodontitis, showed an inverse association between NBK and percent of sites with AL $\geqslant$ 5 mm ($p < 0.05$) and a direct significant interaction between GGTP (> 51 international units) and increasing NBK activity on percent of sites with AL $\geqslant$ 5 mm ($p < 0.05$). **Conclusions:** In AA males with excessive alcohol use, neutrophils show depressed NBK. Depressed NBK was not associated with loss of periodontal attachment in this population. Furthermore, AA males with excessive alcohol use and uncompromised neutrophil function are at greater risk of periodontal tissue damage.

Key words: Periodontitis, periodontal disease, human, neutrophils, alcoholism

Introduction

Alcohol dependence is a chronic and often progressive disease affecting millions of Americans (Grant *et al.,* 2004; Compton *et al.,* 2007; Hasin *et al.,* 2007). Symptoms include a strong need to drink despite negative consequences. The disease is influenced by genetic and environmental factors and is associated with serious social and health problems.

Several studies and case reports indicated increased periodontal problems among heavy alcohol users and abusers. Case reports document the presence of gingival inflammation and increased probing depth among alcoholics (Larato, 1972; King *et al.,* 1973). Shizukuishi *et al.* (1998) reported an association between amount of alcohol consumption and periodontal disease in Japanese factory workers. Tezal *et al.* (2001) reported that alcohol consumption in a group of non-abusers was associated with increased severity of periodontitis. Pitiphat *et al.* (2003) suggested that alcohol consumption is an independent modifiable risk factor for periodontitis.

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The etiology and mechanisms of periodontitis among alcoholics are not known. The role of bacteria in initiating periodontal disease is well established (Darveau *et al.,* 1997; Socransky *et al.*, 1997; Socransky *et al.,* 1998). However, the development and progression of the periodontitis lesion is dependent on the host response (Kornman *et al.,* 1997; Page and Kornman, 1997; Page *et al.,* 1997; Page, 2002). Individuals with immune abnormalities, in particular neutrophil dysfunction, are at greater risk for developing periodontitis (Van Dyke *et al.,* 1984; Deas *et al.,* 2003). Alcohol abuse has been associated with changes in the host response including depressed neutrophil function, depressed lymphocyte function (Cook *et al.,* 1996; Sacanella *et al.,* 1998; Sacanella *et al.,* 1999; Spies *et al.,* 2004) and depressed natural killer (NK) cell activity (Charpentier *et al.,* 1984; Kronfol *et al.,* 1993; Cook *et al.,* 1997).

Previously we showed that alcoholics without medical complications have normal immune cell function, with the exception of altered neutrophil function in male alcoholics (Schleifer *et al.,* 1999). We also reported increased level of periodontal attachment loss in male alcoholics (Khocht *et al.,* 2003). We hypothesized that neutrophil dysfunction associated with alcohol abuse may explain the susceptibility to

periodontitis among male alcoholics. The objectives of this study were to investigate the relation between neutrophil dysfunction and loss of periodontal attachment in male alcoholics.

Materials and methods

Parent study

Subject recruitment and clinical evaluations were previously described (Schleifer *et al.,* 1999; Khocht *et al.,* 2003). To summarize, alcoholic subjects were recruited from a patient population presenting for evaluation and treatment at a university hospital ambulatory alcohol treatment center (ATC) in Newark, New Jersey. The majority of patients attending the ATC were indigent, from 30-60 years of age, more than 90% African American, with a 3:1 male:female gender distribution. Initial screening at ATC referred to the study only English-speaking subjects. Those showing evidence of disabling or life-threatening medical disorders were not referred, including those with known neoplastic diseases or AIDS. Patients presenting with injecting drug use or other primary non-alcohol substance abuse were also not referred. A total of 1870 patients were screened at ATC. Only 320 subjects met the inclusion criteria (English-speaking African American adults receiving care at ATC and free from life-threatening medical disorders) and were referred to the study. Non-alcoholic subjects consisted of inner city residents recruited from community programs (including indigent persons receiving assistance from local churches and other centers) and personal referrals.

All subjects read and signed an informed consent form approved by the UMDNJ-New Jersey Medical and Dental Schools Institutional Review Boards. Health, drug use and cigarette smoking history were taken. This was followed by physical evaluation by a physician. Blood chemistry and urine toxicology tests were performed. Nutrition status was assessed using retinol binding protein assays. Subjects with systemic health problems with potential effects on the immune system or periodontium (e.g., alcoholic hepatitis, alcoholic cirrhosis, AIDS or diabetes) were excluded.

All subjects entered underwent psychosocial assessments by trained interviewers meeting inter-rater reliability criteria to diagnose substance dependence. The symptoms of dependence were determined according to the Diagnostic and Statistical Manual of Mental Disorders, Third Edition Revised (DSM-III-R) (1987), using the Structured Clinical Interview for DSM-III-R (SCID), designed to enable trained interviewers to make substance dependence diagnoses according to DSM-III-R. The SCID established alcohol dependence and history of abuse of other substances. Alcohol exposure was assessed by blood alcohol levels and gamma glutamyl transpeptidase (GGTP) blood levels. For representative recent and long-term time frames, history of type and volume of alcohol consumed was taken. A modified quantity-frequency-variability index was used to estimate equivalent total alcoholic drinks (Schleifer *et al.,* 1999).

Dental study

A total of 102 subjects consented to participate in the periodontal evaluations. Thirty-four subjects were disqualified for not completing all study examinations and three subjects were excluded for being edentulous. Entered into the study were a total of forty DSM-III-R-verified alcoholic dependent subjects, either exclusively (n = 10) or with additional cocaine abuse (n = 30), and a matched comparison group of 25 non-alcoholic subjects, 14 of whom were cocaine abusers. Subjects in both groups were all African Americans. Non-alcoholic subjects were matched to alcoholic patients on variables of age and sex.

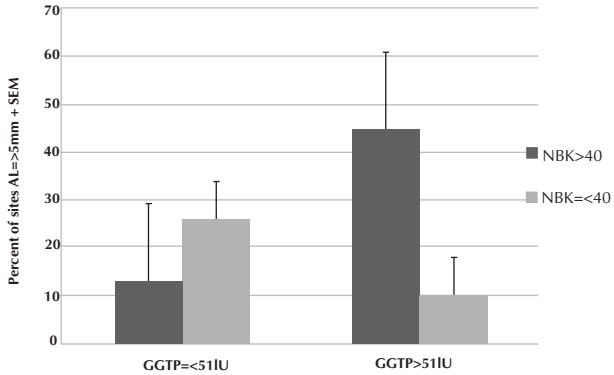
On the day of their dental examination, all subjects answered a dental health questionnaire. This was followed by a comprehensive oral/periodontal evaluation, including probing measurements. All dental exams were performed by examiner AK. The examiner was calibrated and standardized in the use of the clinical evaluation measures employed in the study (Marks *et al.,* 1993; Khocht *et al.,* 1998). The examiner recorded the Löe and Silness gingival index (GI) (Löe, 1967) around all teeth present. Each tooth was scored at six sites: mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual. The teeth were then disclosed with D and C Red No. 28 dye and the Quigley Hein plaque index (Quigley *et al.,* 1962) on the same six surfaces was determined. Surfaces with large restorations and teeth with crowns were not scored.

A conventional periodontal probe with Williams markings (PQ-OW, Hu-Friedy) was used for all probing measurements. Probing depth (PD) was measured at six sites per tooth: mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual. The probe was inserted parallel to the long axis of the tooth on the buccal and lingual surfaces. Interproximally, the probe was placed with slight angulation, as close to the contact area as possible. At the gingival margin the reading was taken to the nearest millimeter. The position of the gingival margin (GM) to the cementoenamel junction was recorded at the same six sites per tooth to the nearest millimeter. A (-) sign was given when the gingival margin was coronal to the cementoenamel junction and a (+) sign when it was apical. Attachment levels were calculated according to the formula AL = PD + GM. For all the aforementioned examinations, only fully erupted teeth were used, except third molars were not included. The examiner was blinded in regard to alcohol dependence diagnosis.

Immune cell enumerative/functional techniques

Blood samples for the immunologic assays were obtained in late morning or early afternoon. Samples were collected into heparinized tubes (preservative-

Figure 1. Attachment level (mean percent of sites with AL ⩾ 5 mm) by alcohol exposure (GGTP level) and dichotomized NBK activity. The bars represent mean percent of sites with AL ⩾ 5 mm. The whiskers indicate the standard error of the mean. Analysis of variance showed significant differences between the groups; p = 0.02. Post-hoc analysis (Tukey's test) showed that the percent of sites with AL ⩾ 5 mm is higher in individuals with a combination of high GGTP (> 51 IU) and high NBK (number of bacteria killed > 40) than in individuals with low GGTP (⩾ 51 IU) and high NBK (number of bacteria killed > 40); p = 0.05.



free). Total white blood cell and differential counts were performed by standard techniques. After separation of the neutrophils from whole blood by centrifugation on a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Piscataway, NJ), neutrophil function was assessed by examining both the phagocytic and killing ability (at 1 and 2 hrs) of neutrophils for *Staphylococcus aureus* according to the method of Weir (1978) with modifications (Georgescu *et al.,* 1987). All neutrophil functional assays were carried out the same day as venipuncture.

The clinical periodontal data of the entire subject group were previously reported (Khocht *et al.,* 2003) and the immune data for 10 exclusively alcoholic subjects were reported as part of a previous study (Schleifer *et al.,* 1999). For the purposes of this report, 32 male subjects with neutrophil measures were used.

Statistical analysis

Statistical analysis was performed with JMP v.9 statistical software package (SAS Institute Inc., Cary, NC). Regression analysis was used to examine the relation between alcohol use and neutrophil function, and the interaction of neutrophil function and alcohol use on periodontal measures. The average PI, GI, AL, and percentage of sites with periodontal attachment loss ⩾ 5 mm were calculated for each subject. The main measure of extent and severity of periodontal tissue damage used in this analysis was the percent of periodontal sites per person with attachment loss ⩾ 5 mm.

Results

Table 1 summarizes the demographic, alcohol/cocaine/cigarette use, periodontal and neutrophil measures of the 32 male subjects reported in this analysis. All subjects were African American and their age ranged from 24 to 61 years. Sixty-six percent of subjects were alcohol dependent, and 40% had GGTP levels > 51 international units (IU), indicating high alcohol consumption. Most of the subjects were cocaine users (72%) and cigarette smokers (84%). Plaque levels were high (average PI = 2.65), suggesting poor oral hygiene. The percent of subjects with loss of periodontal attachment ⩾ 5 mm in 10% or more of examined sites was 60%, indicating a high prevalence of periodontitis. Blood neutrophils counts were within normal levels, averaging 3 x 10^{-6} cells/ml. The number of bacteria ingested ranged from 10-466 cells, and number killed ranged from 7-84. The number of bacteria killed correlated with the number of bacteria ingested ($r = 0.34$, $p = 0.005$).

Distributions of percentage of sites with periodontal attachment loss ⩾ 5 mm and phagocytosis tests were skewed and were rank transformed before further analysis.

Association between GGTP and neutrophil measures

Multiple regression analysis, adjusting for age and periodontal status, showed an inverse association between GGTP and neutrophil bacterial killing (NBK; beta weight = -0.48, $p = 0.04$). This indicates that excessive alcohol use is associated with diminished neutrophil function.

No associations were found between GGTP with either neutrophil counts or neutrophil phagocytosis. No associations were found between neutrophil measures and either cocaine or cigarette use.

Association among GGTP, NBK and periodontal measures

A series of multiple regression models were used to examine the association between GGTP, NBK and percent of sites with AL ⩾ 5 mm. In the first model ($R^2 = 0.32$) we examined the effects of GGTP, NBK and

Table 1. Summary of demographic, periodontal and neutrophil data of the 32 African American male subjects presented in this report.

Demographics	
Age [mean (SD)]	41.66 (9.24)
Percent cigarette smoking	84%
Percent cocaine use	72%
Percent alcohol dependence	66%
Percent GGTP > 51 IU	40%
Periodontal measures	
PI [mean (SD)]	2.65 (0.67)
GI [mean (SD)]	1.04 (0.16)
PD [mean (SD)] mm	2.85 (0.51)
AL [mean (SD)] mm	3.38 (1.0)
Percent of sites with AL ⩾ 5mm [mean (SD)]	21.12 (23.16)
Neutrophil measures	
Blood cell counts: Cells/ml x $l0^{-6}$ [mean (SD)]	3.00 (1.56)
Phagocytosis test: Number of bacteria ingested at beginning of killing incubation time, reported as mean (SD).	100.49 (95.08)
Bacterial killing test (NBK): Number of bacteria killed, average of two reading times, reported as mean (SD)	47.34 (17.65)

AL, attachment loss; GGTP, gamma-glutamyl transpeptidase; IU, international units; NBK, neutrophil bacterial killing; SD, standard deviation

their interaction on percent of sites with AL ⩾ 5 mm without any adjustments. Neutrophil bacterial killing showed an inverse association with percent of sites with AL ⩾ 5 mm, albeit a non-significant one (beta-weight = -0.41, $p < 0.48$). The interaction of GGTP with NBK (beta-weight = 0.58, $p < 0.05$), and GGTP alone (beta-weight = 0.49, $p < 0.05$), showed direct independent associations with percent of sites with AL ⩾ 5 mm. The interaction showed a contrasting NBK effect between low alcohol consumers (GGTP > 51 IU) and high alcohol consumers (GGTP ⩾ 51 IU). In individuals with low alcohol consumption NBK inversely associated with percent of sites with AL ⩾ 5 mm. On the other hand, in individuals with high alcohol consumption NBK directly associated with percent of sites with AL ⩾ 5 mm. Figure 1 represents these findings graphically in a dichotomous presentation of NBK activity by GGTP levels.

In a follow-up regression model we adjusted for known risk factors of periodontitis: age, cigarette smoking and plaque levels. Adding these factors greatly improved the model fit ($R^2 = 0.57$) and revealed the independent inverse association between NBK and percent of sites with AL ⩾ 5 mm (beta-weight = -0.61, $p < 0.05$). The interaction between GGTP and NBK maintained its independent association with percent of sites with AL ⩾ 5 mm (beta-weight = 0.62, $p < 0.05$) with similar effects as in the unadjusted model. Gamma-glutamyl transpeptidase lost its independent association with percent of sites with AL ⩾ 5 mm, and plaque levels showed an independent direct association with percent of sites with AL ⩾ 5 mm (beta-weight = 0.46, $p < 0.05$). This suggests that plaque levels mediated the independent direct association between GGTP and percent of sites with AL ⩾ 5 mm noted in the unadjusted model.

No associations were found between NBK and GGTP with gingival inflammation and periodontal probing depth. Cocaine use showed no associations with periodontal measures (data not shown).

Discussion

A multitude of studies have reported altered immune function in alcohol-dependent patients (Charpentier *et al.,* 1984; Cook *et al.,* 1990; Kronfol *et al.,* 1993; Cook *et al.,* 1996; Cook *et al.,* 1997; Cook, 1998; Sacanella *et al.,* 1998; Sacanella *et al.,* 1999; Spies *et al.,* 2004). Previously, we (Khocht *et al.,* 2003) and others (Larato, 1972; King

et al., 1973; Shizukuishi *et al.,* 1998; Tezal *et al.,* 2001; Pitiphat *et al.,* 2003) reported increased periodontal pathology in alcoholics. Even though periodontitis is initiated by specific bacterial pathogens, the host immune response determines the pathogenesis of disease (Page and Kornman, 1997; Page *et al.,* 1997). White blood cells orchestrate the immune response (Page and Kornman, 1997; Page *et al.,* 1997). In this study we investigated the effect of high levels of alcohol exposure on neutrophils and the consequential effects on the periodontium in a group of alcohol-dependent male patients without medical disorders.

A unique aspect of this study is the stringent selection criteria used to include only alcohol users without systemic illness. Each potential subject underwent comprehensive clinical medical evaluations and blood tests to exclude individuals with systemic conditions that may affect the immune system and the periodontium. All subjects entered in the study were medically healthy and free from systemic disorders such as immunodeficiency diseases, diabetes, renal disease and liver disease.

Neutrophils represent the first line of defense against infections and are considered the principal cells of the innate (non-adaptive) immune system. They are the most numerous of all the white blood cells, representing 50% - 70% of total circulating leukocytes. Polymorphonuclear leukocytes (PMN) numbers in peripheral blood of alcoholic patients are dependent on the stage of alcoholic liver disease. In alcohol-dependent patients with early stages of liver disease, PMN numbers in peripheral blood tend to be elevated and liver tissues are heavily infiltrated with neutrophils (Cook *et al.,* 1990; Cook, 1998). It is suspected that the neutrophils in liver tissues contribute to liver damage with the release of tissue damaging enzymes (Cook *et al.,* 1990; Cook, 1998). In later stages of alcohol dependence neutrophil numbers in peripheral blood are seriously diminished because of bone marrow suppression (Cook *et al.,* 1990; Cook, 1998).

Neutrophil functional abnormalities previously described in association with excessive alcohol exposure include decreased chemotaxis and bacterial killing (Jareo *et al.,* 1995; Jareo *et al.,* 1996), inhibition of neutrophil migration (Brayton *et al.,* 1970; Gluckman *et al.,* 1978; Avaria *et al.,* 1981; Astry *et al.,* 1983), and decrease in expression of adhesion molecules (MacGregor *et al.,* 1988; Zhang *et al.,* 1998). Data from our study showed decreased neutrophil bacterial killing function with increased alcohol consumption in healthy male alcoholics. A better understanding of how excessive alcohol exposure can influence molecular events inside neutrophil cells may explain the noted depressed bacterial killing function. After exposure to alcohol, the calcium ion concentration inside the neutrophils is shifted, which may alter function and influence transcriptional control (Nilsson *et al.,* 1992; Patel *et al.,* 1996). Feeding alcohol to rats inhibited neutrophil production of reactive oxygen radicals (Jareo *et al.,* 1996).

As would be expected, data from this study showed that in individuals with low alcohol consumption neutrophil bacterial killing played a protective role against periodontal tissue loss. Contrary to our hypothesis, in individuals with high alcohol consumption diminished neutrophil function was not associated with periodontal tissue damage and was inversely related to loss of periodontal attachment. Diminished neutrophil bacterial killing would be associated with decreased release of inflammatory mediators and reduced oxidative stress, thus minimizing tissue damage (Van Dyke *et al.,* 2003). On the other hand, in this population of excessive alcohol users with high plaque levels, in individuals with uncompromised neutrophil function, neutrophil bacterial interactions and associated inflammation/oxidative stress would result in greater tissue damage and would explain the increased loss of periodontal attachment (Kantarci *et al.,* 2003; Johnstone *et al.,* 2007). These findings are in concordance with other reports showing increased periodontal tissue damage in individuals with high neutrophil activity (Shapira *et al.,* 1991; Bender *et al.,* 2006; Khocht *et al.,* 2010; Khocht *et al.,* 2012).

Alcohol-dependent individuals often abuse other drugs (Kaufman 1982; Kamerow *et al.,* 1986; Tam *et al.,* 2000). Also, several studies reported an association between excessive alcohol drinking and cigarette smoking (Attwood *et al.,* 2012; Ward *et al.,* 2012). Many of the subjects included in this report abused cocaine and were cigarette smokers. To investigate the relation between alcohol use and neutrophil function in the presence of cocaine use and cigarette smoking we used multiple regression analysis to control for these variables. In this study, neither cocaine use nor cigarette smoking showed an association with neutrophil function.

In conclusion, healthy AA males with excessive alcohol use show diminished neutrophil function. Diminished neutrophil function does not associate with loss of periodontal attachment in this population. Furthermore, AA alcoholic males with uncompromised neutrophil function and high plaque levels are at greater risk for periodontal tissue damage.

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