Relationship between Oxidative Stress and Oral Disease in a Rural Cambodian Population: Evaluation of Oxidative Stress in a Developing Country

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Abstract
In rural areas of developing countries, rapid aging and reduced life expectancy have been observed, and it has been hypothesized that excess oxidative stress is one cause of rapid aging. Oral disease may contribute to one of the risk factors in the production of oxidative stress. The purpose of this study was to examine the influence of oral disease on oxidative stress in the rural Cambodian population.

Eighty-seven subjects living in the rural area in Mondulkiri Province, Cambodia were examined as a target group, and data were obtained regarding caries experience, probing pocket depth (PD), and bleeding on probing (BOP). The level of oxidative stress was evaluated by the diacron reactive oxygen metabolites (d-ROMs) test.

The results revealed that the level of d-ROMs correlated with their age, the number of healthy teeth (HT), the number of missing teeth (MT), the decayed, missing and filled teeth (DMFT) index, the number of present teeth (PT), the rate of ≥4mm PD, and the BOP rate. In stepwise multiple regression analysis, the BOP rate was the most influential parameter linking to the production of oxidative stress as an independent variable. HT was secondary, followed by the number of filled teeth (FT). A control group, 30 residents of Phnom Penh City within an urban environment, was compared with the target group. The target group had significantly higher levels of oxidative stress, caries experience, periodontal status, and diastolic blood pressure (BP). The findings of this study indicate that periodontal disease may increase the production of oxidative stress, and the data obtained suggest that HT and FT contribute to the reduction of the level of oxidative stress.

Introduction
In rural areas of developing countries, residents have demonstrated a trend toward rapid aging (1), which appears to contribute to shortened life expectancy, and health hazards such as early onset of diseases associated with aging. One possible mechanism underlying the rapid aging is excess oxidative stress.

Oxidative stress has been defined as an imbalance between the production and the elimination of oxidant stress (2). A correlation between oxidative stress and the telomere shortening rate was described (3), and it was reported that some lifestyle-related diseases were induced by oxidative stress, which leads to DNA damage and cell membrane injuries (4). The causes of increased oxidative stress included inflammation, diabetes, ultraviolet rays, radiation, bacterial infection, some drugs, smoking, and energy metabolism, among others (5).

Several prior studies have identified a relationship between oxidative stress and the oral region. A rat model study confirmed that mastication reduces oxidative stress (6), and drugs used in dental treatment such as hydrogen peroxide and sodium hypochlorite produce oxidative stress (7). Gingival fibroblasts from Down syndrome patients, who sometimes experience premature aging and/or early-onset periodontitis, were reported to show high levels of oxidative stress (8). Oxidative stress is significantly higher in periodontitis patients compared to periodontally healthy subjects.
Moreover, it has been shown that periodontal treatment decreases oxidative stress (10, 11). Although there is increasing evidence that periodontal disease contributes to oxidative stress, the prior studies were not systematic investigations, and to the best of the author’s knowledge, no evidence has been published regarding a potential link between caries experience and oxidative stress. Two studies focusing on oxidative stress were conducted in developing countries (12, 13); their authors reported that residents of developing countries had higher levels of oxidative stress. Apparently, however, no evidence has been reported regarding the relationship between oxidative stress and oral disease in developing countries. The identification of any relationship between oral disease and oxidative stress could contribute greatly to community oral health activity in developing countries. Thus, the purpose of the present study was to examine the potential link between oxidative stress and oral disease in rural Cambodian residents.

**Materials and Methods**

**Survey Schedule**

This research was conducted on March 2010, from November 2010 to February 2011, and September 2011 in Mondulkiri Province and Phnom Penh City in the Kingdom of Cambodia.

**Target Group**

The target group was 87 adults (38 males, 49 females, mean age: 36.7 ± 14.3 years, range: 18-71 years) living in Mondulkiri Province (a rural area that is one of the undeveloped economical, administrative, and health service provinces with approximately population of 60 thousand people in Cambodia). They were selected at random from 201 adults (81 males, 120 females, mean age: 33.3 ± 13.5 years, range: 18-71 years) who had joined an oral health program operated by the University of Health Sciences Faculty of Odonto-Stomatology in the Kingdom of Cambodia (UHSOS). The target group subjects were not taking any medications or drugs, and they showed no abnormalities in urinalyses. The Multistix SG-L (Siemens, Munich, Germany) was used for the urinalyses.

**Control Group**

The control group was composed of 30 adult volunteers (14 males, 16 females, mean age: 37.4 ± 10.4, range: 20-58 years) living in Phnom Penh City, the capital city of Cambodia with an approximately population of 2.2 million people. The control group subjects were not taking any medications or drugs, as confirmed by the same urinalysis method used for the target group.

**Target and Control Group Characteristics**

The characteristics of all subjects were charted according to gender, birth date, height, weight, blood pressure (BP), and pulse rate. Body mass index (BMI) was calculated from weight and height (weight [kg]/height² [m²]) (14).

**Examination of Oral Status**

The number of decayed teeth (DT), missing teeth (MT) and filled teeth (FT) was investigated in all subjects to evaluate caries. DMFT index, the number of healthy teeth (HT) and the number of present teeth (PT) were calculated for each subject. Probing pocket depth (PD) was examined for the evaluation of periodontal disease. Six sites per tooth were examined, and the maximum PD value was charted. Active inflammatory PD was examined by bleeding on probing (BOP). The BOP was evaluated as the presence or absence of bleeding at 30 sec after probing. The BOP was determined to be positive in at least one site of the presence of bleeding which was examined in six sites of pocket probing. The rate of ≥ 4 mm PD and the rate of positive BOP were calculated for each subject. In advance of the survey, calibration training was conducted for four Cambodian dentist-examiners, based on the World Health Organization (WHO) Oral Health Surveys Basic Methods (15, 16).

**Examination of Oxidative Stress**

Oxidative stress was evaluated by the diacron reactive oxygen metabolites (d-ROMs) test using an FRAS4 system (Diacron, Grosseto, Italy). The test sample was 20μL of peripheral blood collected from the fingertip. The collection of blood was conducted by only Cambodian dentists. This test is based on the ability of transition metals to catalyze, in the presence of peroxides, the formation of free radicals that are trapped by an alchylamine. The alchylamine reacts, forming a colored radical detectable at 505nm through a kinetic reaction. The determination of free radicals can be made with a normal spectrophotometer. The measurement unit used was the Carrabelle unit (U.CARR). The normal range has been established as 250-300 U.CARR. It has been established that 1 U.CARR corresponds to 0.08mg/dL.
hydrogen peroxide. Less than 300 U.CARR is a normal value (17, 18).

**Statistical Analysis**

All data obtained in this study are presented as mean ± standard deviation. Pearson’s correlation coefficient was used to evaluate the results of the d-ROMs and the parameters involved. A stepwise multiple regression analysis was carried out to detect the degree of association between the d-ROMs level as the dependent variable and the parameters age, HT, DT, MT, FT, rate of ≥4mm PD, BOP rate, BMI, systolic BP, diastolic BP and pulse rate as independent variables. Student’s t-test was used to compare parameters between the target group and the control group. All analyses were performed using software (IBM SPSS 20.0, IBM Corp., Armonk, N. Y.). A p-value < 0.05 was considered significant.

**Ethical Approval**

This study was approved by the Ethical Committee of Nihon University School of Dentistry at Matsudo in Japan (EC10-024) and also approved by the UHSOS.

**Results**

The characteristics of subjects and the comparison between the target group and the control group are shown in Table 1 and Fig. 1. Significant between-group differences were observed for DT (p < 0.001), MT (p < 0.05), FT (p < 0.001), DMFT (p < 0.05), rate of ≥4 mm PD (p < 0.001), BOP rate (p < 0.001), diastolic BP (p < 0.01) and d-ROMs (p < 0.001). No significant differences between the target and control groups were found in age, HT, PT, height, weight, BMI, systolic BP and pulse rate.

The results of the Pearson’s correlation coefficient are shown in Tables 2 and 3. The level of d-ROMs in the target group significantly correlated with age (r: 0.24, p < 0.05), HT (r: -0.33, p < 0.01), MT (r: 0.24, p < 0.05), DMFT (r: 0.30, p < 0.01), PT (r: -0.23, p ≥ 0.01), rate of ≥4 mm PD (r: 0.28, p < 0.01), and BOP rate (r: 0.43, p < 0.001). The level of d-ROMs in the control group did not significantly correlate to any of the parameters.

The degree of association between the d-ROMs results and the parameters was examined using a stepwise multiple regression. The BOP rate, HT, and FT data were included in a regression model by the stepwise method. Age, DT, MT, rate of ≥4 mm PD, BMI, systolic BP, diastolic BP and pulse rate were excluded from the regression model. The BOP rate (standardized β: 0.40, p < 0.001) was the most influential factor in the increase of the level of d-ROMs. HT (β: -0.30, p < 0.01) and FT (β: -0.23, p < 0.05) were
negatively related to the level of d-ROMs. The results of the stepwise multiple regression are shown in Table 4.

### Discussion

Cambodia has not yet been well developed, including aspects of the social infrastructure such as traffic routes, the healthcare system, and the education system (19). The country’s undeveloped healthcare system has become one of the reasons for lower life expectancy (61 years) and an increased infant mortality rate (68/1000) (20). Cambodia’s Human Development Index is 0.50 (124th of 169 countries among the UN members) (21). A great numbers of Cambodia’s educated population were slaughtered in the civil war during the Pol Pot period, which began in 1975 and lasted about 4 years (22). The loss of manpower was a main cause of the delay in the construction of medical infrastructure. The under-development of the medical infrastructure
in rural areas is even more serious than in urban areas. Mondulkiri Province, the target area of the present study, is 400 km away from Phnom Penh City and located in the northeastern part of Cambodia. It is said that it is one of the poorest rural provinces of Cambodia.

One of the causes of the rapid aging and reduced life expectancy in rural areas of developing countries is hypothesized to be an excess oxidative stress. The “free radical theory” is currently the most influential theory among the various theories of aging. The free radical theory posits that aging is accelerated by oxidative stress produced by reactive oxygen species, which are generated from the processes of inflammation, diabetes, ultraviolet rays, radiation, bacterial infection, some drugs, smoking, and energy metabolism, among others (5). The level of oxidative stress is higher in patients with cancer, disease-infarct, hypertension, obesity, atherosclerosis, diabetes, and periodontal disease (9-11, 23). In addition, differences in the level of oxidative stress between geographic regions have been reported (12, 13).

Biomarkers of oxidative stress have been widely researched (8, 17, 24-26). The d-ROMs test which was suitable for field research was used in the present study. The d-ROMs test is assumed to be a reliable and highly reproducible method (17). The results of the present study are relevant to the potential correlation between d-ROMs data and oral diseases. The data are suggesting that periodontal disease is a factor contributing to oxidative stress. This finding is similar to those obtained in syngeneic studies (9-11). Caries experiences (MT, DMFT) were also suggested as one of factors contributing to increased oxidative stress. In contrast, the present healthy teeth indicate that the remaining no-caries is a factor in decreased oxidative stress. This may be the first study to demonstrate a relationship between caries experience and oxidative stress.

Nagata et al. (27) reported that age did not correlate with oxidative stress in healthy subjects. Hirose et al. (28) reported that age was negatively correlated with oxidative stress in male subjects at a physical checkup. The age of the subjects in the present study was also significantly correlated with the d-ROMs results. In the stepwise multiple regression analysis, age was not selected as an independent variable. The link between age and oxidative stress remains controversial.

The BOP rate was found to be the most influential factor to generate oxidative stress as an independent variable in this study; HT was secondary, followed by FT. It has been suggested that BOP indicates the prevalence of inflammation to be the most affecting parameter of all the parameters on the level of oxidative stress. Continuing chronic inflammation from periodontal disease in local periodontal tissue and the influence of the systemic condition from the bacteremia of periodontal pathogenic bacteria were suggested to be related to the generation of oxidative stress. It is possible that oxidative stress is decreased by advanced immune functions based on adequate nutrition and mastication by healthy teeth, and it was confirmed that mastication reduced oxidative stress in a rat model (6). Nasu et al. reported that the maintenance or recovery of sufficient chewing ability was related to a longer, active life expectancy (29). It was suggested that the presence of healthy teeth decreases oxidative stress and may be related to active life expectancy. But, to the best of the author’s knowledge, no evidence has been published regarding oxidative stress in humans as decreasing in accord with mastication ability.

In the present study, since the target area has only two dentists and no dental technician, residents cannot receive dental prosthesis for MT. Actually, few residents have received restorative treatment, but residents have never received prosthesis treatment for MT and have not recovered their mastication ability.

FT (filled teeth) is normally a negative parameter of caries experience in developed countries, but in the present study, in rural areas in developing countries, filled teeth contribute to a decrease in oxidative stress. People living in rural areas and suffering from dental caries in Cambodia cannot receive dental treatment due to the economic conditions and scarcity of dentists. Only a small percentage of the rural population has received dental treatment. It has been speculated that a high standard of living tends to reduce oxidative stress because people with that standard of living are relatively high-income earners, and their mastication ability has been maintained by appropriate medical and social services.

In the present study, the target group of rural residents was compared with a control group living in an urban environment with a relatively advanced living situation and healthcare system. The target group had significantly higher levels of the d-ROMs results. The parameters of caries experience, periodontal disease and diastolic BP were
also significantly higher in the target group when compared with the control group. Miyata et al. (30) and Amarasena et al. (31) reported that residents living in a rural area in Cambodia had severe oral disease, and the periodontitis was significantly worse than that of the studies’ subjects in Phnom Penh City. According to those studies, poor living conditions such as an inadequate supply of safe water, improper toilet facilities, poor nutrition, and the risk of living in proximity to livestock were closely associated with severe periodontal inflammation compared to the advanced living conditions found in Phnom Penh City. In the present study, periodontal disease demonstrated almost the same results. However, the factors contributing to periodontal disease in rural areas remains controversial. There are several limitations to the present study. The present study was cross-sectional, and cohort studies are needed. In addition, studies that examine therapeutic interventions will be very important to clarify the relation between oral disease and oxidative stress.

The systemic status of subjects was detected only by blood pressure measurement and urinalysis as part of the screening for systemic disease. Biochemical blood tests and bacteriologic examinations would provide more precise information, but such tests and examinations were not conducted because of equipment limitations, the lack of necessary power sources, and the difficulty of sample preservation.

In conclusion, these results demonstrate that oral disease has a significant link to increased oxidative stress, and that the presence of healthy teeth contributes to decreased oxidative stress. The amelioration of oral disease may reduce the level of oxidative stress and improve the systemic condition. The present results underscore the importance of maintaining good oral hygiene. Further elucidation of the mechanisms underlying the link between oral disease and oxidative stress is expected.

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