Original article

The effects of smoking habits on the salivary occult bleeding test

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Abstract

It is well-known that salivary occult blood test shows frequently false negative among heavy smokers with periodontal diseases. This makes a major defect of salivary occult blood test when we apply the test as a screening method for periodontal ailments. It is very serious defect especially in Japan where approximately 50 % of male are regarded as smokers. It is urgent need to fulfill well-designed epidemiological studies to answer whether salivary occult blood test is a useful tool assessing periodontal problems among subjects including smokers. To answer this question, we investigated among 1,616 workers in a factory the relationship between periodontal diseases and salivary occult blood test or Community Periodontal Index (CPI) and examined the effect of smoking habits on the correlation.

The analysis revealed that when CPI was applied, the odds ratio for the non-smokers was the lowest and those were elevated for ex-smoker and current smoker, concomitantly, as well as increased by a number of cigarettes per day and duration of smoking habit. When salivary occult blood tests were applied, the odds ratio showed reverse trends among those subgroups. A statistical significant correlation was observed between salivary occult bleeding test and CPI for non-smoker groups of both sex, and only male smokers, while difference was smaller among male smokers.

In conclusion, salivary occult blood test is a useful screening method when applied for non-smokers. The false negative results are frequent among individuals who smoke many cigarettes or for many years.

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《**Key words**》 salivary occult bleeding test, periodontal disease, smoking habit, community periodontal index (CPI)

Ⅰ. Introduction

In the past, it was believed that periodontal disease constituted a major cause for tooth loss and it was a disease entity limited to one's oral cavity. During the latter half of the 1990s, however, studies on the relationship between periodontal and systemic diseases was actively investigated; and the field of oral medicine achieved such rapid advances that a proposal was made in the United States to establish an

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independent medical discipline called periodontal medicine. Subsequently, it was elucidated that periodontal disease is not only responsible for tooth loss: it also affects the development of systemic diseases, such as diabetes mellitus[1], cardiovascular disease[2] et al.. Thus we have come to recognize that preventing periodontal diseases from developing is important, not only for maintaining a healthy oral environment but also to sustain one's overall health. In "Healthy Japan 21", periodontal diseases are included in the ranks of disease entities traditionally considered to be of more serious consequences (such as cerebral apoplexy and cardiac diseases).

In this environment, it is deemed vital that an appropriate screening test be established to prevent periodontal diseases. The conditions that are commonly considered necessary for a health screening test[3] are: (1) high validity or reliability; (2) ease in application; (3) non-invasiveness; and (4) low cost. For the diagnosis of these diseases, clinical indices[4], such as the PMA Index, Periodontal Index (PI), Bleeding Index (BI) and Attachment Loss (AL) with special references to gingival inflammatory syndrome and periodontal pockets, as well as radiographic examinations of the alveolar bone are cited. All these are conducted at an oral examination by dentists or dental hygienists. In addition, they all require time and their results are hampered by inter-observer differences. Therefore none are suitable for screening when a large number of individuals are to be examined. On the other hand, saliva has been cited as the test material in a procedure that meets conditions (1)-(4) cited above and that withstands subjective and scientific analysis[5]. Saliva can be collected easily and non-invasively; and it is a diagnostic material that can be used effectively to analyze for various disease conditions and symptoms. A periodontal disease screening test using salivary occult bleeding test has been frequently incorporated into dental health examinations for adults at the local community level or at one's place of work[6].

It has been reported that when an inflammation develops in the periodontal tissue, a hemorrhage from the gingival crevice or pockets may allow the detection of occult blood in the saliva[7]. It is known that the amount of occult blood increases as the inflammation progresses. The salivary occult bleeding test is based on this inflammatory reaction and is therefore considered to satisfy most of the conditions stipulated for a screening test. On the other hand, it has been reported that smokers, in contrast to non-smokers, tend to bleed less from the gingiva when it is probed. At an actual community mass screening, one encounters cases of advanced periodontal diseases in spite of negative results obtained from a salivary occult bleeding test. In other words, it is suspected that a large number of smokers with abnormal periodontal conditions (those suffering from periodontal diseases) display negative results from the salivary occult bleeding test (i.e., exhibiting false negative responses)[8]. In view of the fact that about 50 % of the men in Japan smoke, highly accurate epidemiological studies are necessary to define the relationship between the salivary occult bleeding test and one's smoking habit. In Japan, the survey methods are beset by problems, such as the number of test subjects and selection bias, so the epidemiological findings have thus far been insufficient. To overcome these shortcomings and define the effects of smoking on the salivary occult bleeding test while striving to overcome the aforementioned problems, the current study was conducted on 1,616 factory workers.

Ⅱ. Methods

1. Subjects

They were employees of a chemical plant located in Takaishi, Osaka, Japan. Among the employees who received routine health examinations in 1998, those who satisfied conditions 1) through 4) were selected: 1) Men and women between the ages of 20 to 59 years; 2) Those who did not require more detailed examinations other than a routine health examination; 3) Those who were not given a diagnosis of chronic diseases at the routine examination (such as cardiac disease or diabetes mellitus); 4) Those who did not report an abnormality, such as fatigue or a common cold on the day of the routine health examination.

Most operated the computer-controlled equipment and were not at risk of being exposed to toxic chemicals. The number of individuals ultimately selected were 1,616.

Table 1 shows the number of subjects by gender and age.

Table 1 The study population of subjects by gender and age

Age	Male n(%)	Female n(%)	Total n(%)
20-29	379(25.7)	56(39.5)	435(26.9)
30-39	293(19.9)	33(23.2)	326(20.2)
40-49	520(35.3)	33(23.2)	553(34.2)
50-59	282(19.1)	20(14.1)	302(18.7)
Total	1,474(100.0)	142(100.0)	1,616(100.0)

2. Survey on smoking habits

For the survey on smoking habits, a self-responding questionnaire was distributed among the participants and the completed sheets were retrieved on the same day. The questions included “having or not having a smoking habit”, “the number of cigarettes smoked per day”, and “duration of smoking”.

3. Dental examination

1) Measurement of occult blood in saliva[8)]

The test subjects were instructed to refrain from eating, drinking, or cleansing their oral cavity starting 2 hours prior to the scheduled oral examination; and were instructed to collect an appropriate amount of resting saliva just before the oral examination. After collecting a salivary specimen, “Salivaster-Bld.” (Showa yakuhin kako. LTD.) was used immediately for semi-quantitative determination of the occult blood in the saliva. A single examiner read all the test results. Based on the standard coloration for hemoglobin concentrations of 0.4 , 1.0 , and 2.6 mg/dl, the quantity of occult blood was rated at one of 6 levels, i.e., scored 0 to 5 .

2) Measurement of Community Periodontal Index (CPI)[9)]

The dental examinations were conducted by an experienced dentist and 3 dental hygienists. The subject sat on a simple chair designed for dental examinations, with his head positioned securely. A light was arranged to permit accurate examination. About 5 minutes were allocated for each examination.

The progression of periodontal diseases was expressed by using the CPI, which was based on a method proposed by Ainamo, et al. Specifically, the oral cavity was divided into 6 sections and the periphery of one or two teeth, representing each section, was examined with a Periodontal Probe (Yamaura, Inc.) designated by WHO. The clinical findings of these representative teeth in each section were expressed in numerals: the severest findings was assigned to that section. The CPI values were: 0 , unremarkable; 1 , gingival bleeding during or after probing; 2 , dental calculus; 3 , a periodontal pocket depth of 4 mm or 5 mm; 4 , a periodontal pocket depth of 6 mm or more. When a

representative tooth was missing, a substitute tooth was selected for evaluation. If all the teeth were missing, the subject was excluded from the evaluation. In this study, the mean of the CPI score among the 6 sections was used as the numeral representing the degree of progression of periodontal disease in that subject.

4. Statistical analysis

The association between smoking habits and value of CPI and saliva occults blood was analyzed by employing ANOVA. The relationship between smoking habits and the extent of periodontal diseases was determined as follows: the progression of periodontal disease was assigned to one of 3 categories (CPI of 0 , 1 , and 2 ; CPI of 3 ; and CPI of 4), which was used as an independent variable. After adjusting for age, a logistic regression analysis was performed[10] . For statistical analysis, the Macintosh Statview Ver. 5.0 Computer Program was used (SAS Institute Inc., Berkeley, USA). The significance level was set at $p<0.05$.

5. Informed consent and maintaining privacy for subjects

To protect the human rights of the participants, informed consent was obtained. On the same occasion, the purpose of the study was clearly described. They were also advised that the results would only be used to protect the health of the employees of the plant and the method, particulars of the questions posed, and risks were thoroughly explained. The prospective respondents were urged to participate and told that their participation was completely voluntary, their privacy would be thoroughly respected, and the organizers of the study were prepared to answer any questions they might have about the survey.

To maintain the privacy of the respondents, a code was assigned to each individual for the statistical analysis. This would prevent identification of individuals when restrictions on the data evaluation were lifted or the results were published.

Ⅲ. Results

1. Smoking habits in subjects

The subjects were grouped by gender and their smoking habits are shown in Table 2. There were 55.7 % smokers among men and 7.0 % among women. Those smoking more than 21 cigarettes per day were 33.3 % and 0 % for men and women, respectively. Among male smokers, 42.8 % have been smoking for more than 20.1 years.

Table 2 Smoking habits by gender

Variables	Male n(%)	Female n(%)	Total n(%)
Cigarettes smoking			
Non-smoking	500(33.9)	131(92.3)	631(39.1)
Quit smoking	153(10.4)	1(0.7)	154(9.5)
Smoking	821(55.7)	10(7.0)	831(51.4)
Number of cigarettes/day			
1-10	90(10.7)	9(90.0)	99(11.6)
11-20	470(56.0)	1(10.0)	471(55.5)
≧21	279(33.3)	0(0.0)	279(32.9)
Duration of smoking habit (years)			
0.1-10	237(28.2)	7(70.0)	244(28.7)
10.1-20	243(29.0)	1(10.0)	244(28.7)
≧20.1	359(42.8)	2(20.0)	361(42.6)

2. Salivary occult bleeding test and CPI by smoking habits

The results of the salivary occult bleeding test were stratified by smoking habit, number of cigarettes smoked, and the duration of smoking (see Fig. 1-1). No statistically significant differences were noted.

The CPI, stratified in a similar manner, is indicated in Fig. 1-2. Compared with non-

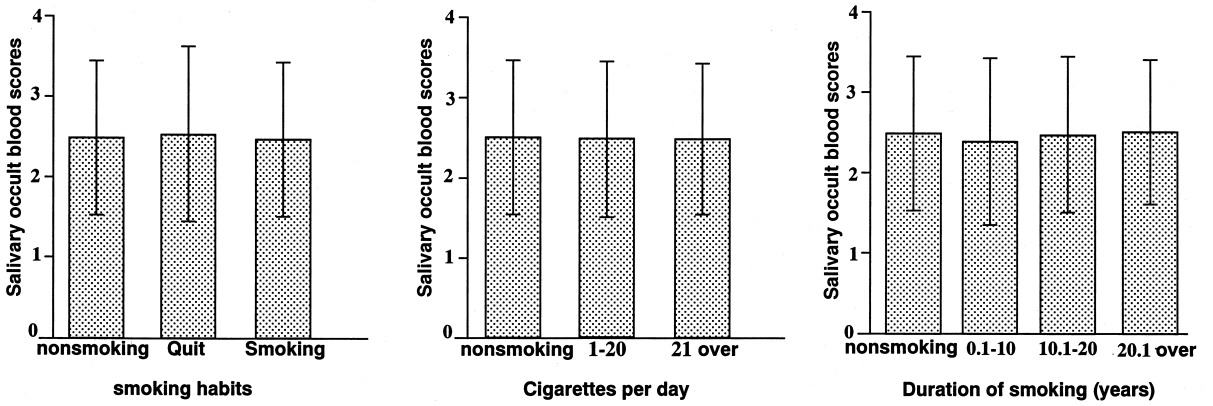

Fig. 1-1 Mean of the Salivary occult blood score and smoking habits

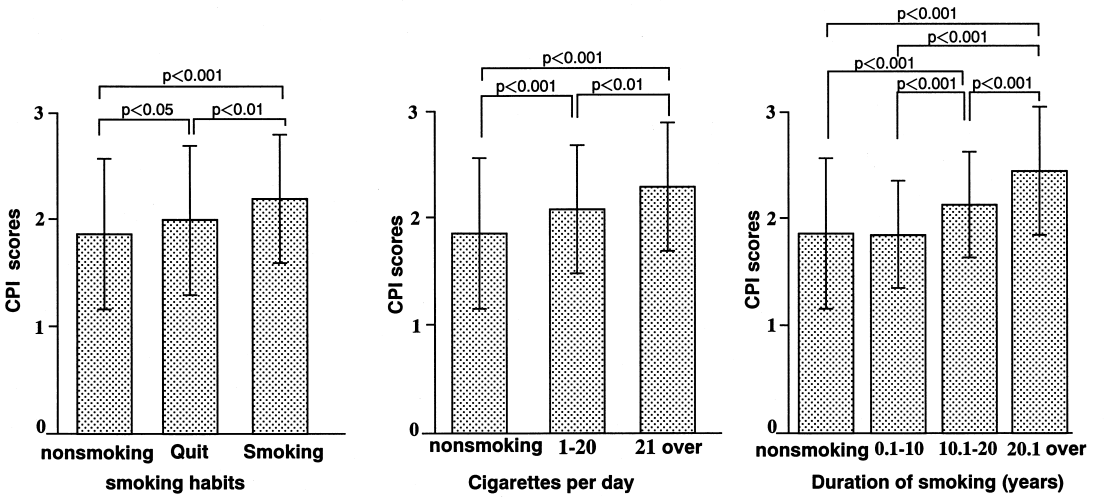

Fig. 1-2 Mean of the CPI score and smoking habits

smokers, the CPI of the smokers was high, the difference between the two groups being statistically significant. With increases in the number of cigarettes smoked and the duration of smoking, the CPI rose with a statistically significant difference.

3. Periodontal disease by smoking habits

Using the results of the salivary occult bleeding test and the CPI as indices for periodontal diseases, the odds ratios for developing periodontal diseases were computed by using logistic regression analyses for the smoking habit, number of cigarettes smoked, and the duration of smoking (Fig. 2). When the CPI was used, the odds ratio for developing a periodontal disease was lowest for the group of non-smokers, followed by the smoking cessation group, and highest for the group of current smokers. There were mounting tendencies for the odds ratio and the probability to develop periodontal diseases in proportion to the increases in the number of cigarettes smoked and the duration of smoking. When the results of the salivary occult bleeding test were applied, the odds ratio

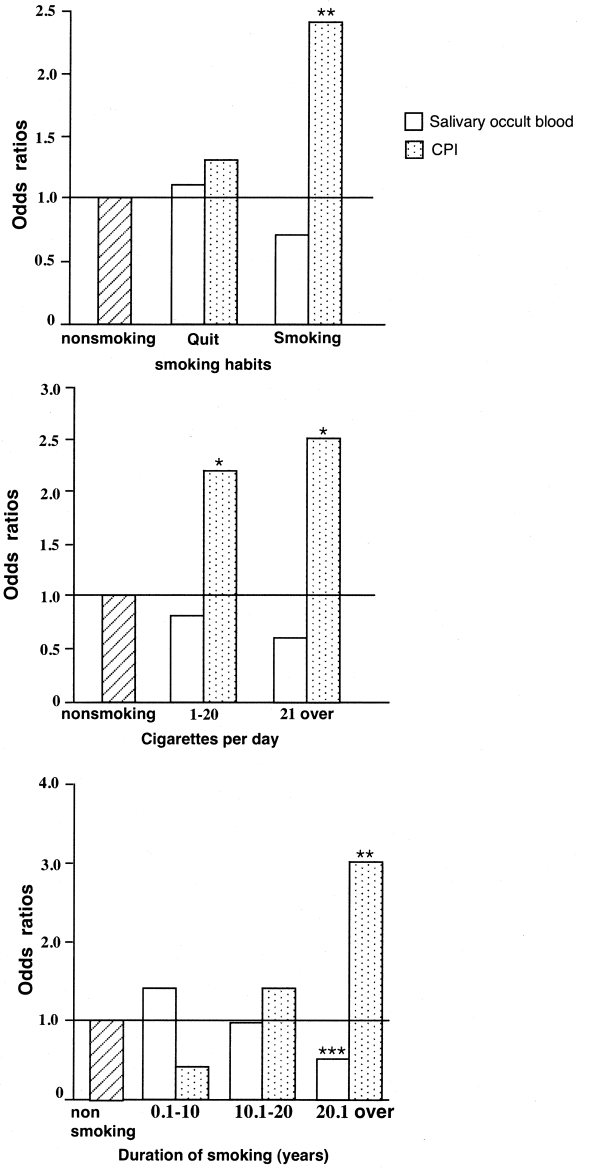

Fig. 2 Odds ratios of the CPI score and salivary occult blood scores by smoking habits

Ajusted for age *: p<0.05 **: p<0.01 ***: p<0.001

for developing periodontal diseases was generally the reverse of the aforementioned tendency associated with the CPI. This time, both the odds ratio and the probability for developing periodontal diseases appeared to be lower as the number of cigarettes smoked and the duration of smoking increased.

4. Relationship between Salivary occult bleeding test and CPI by smoking habits

Stratified by gender and smoking habits, the correlation between the results of the salivary occult bleeding test and CPI is shown in Fig. 3. In the group of non-smokers, statistically significant correlations existed between the results of the salivary occult bleeding test and CPI for both sexes. Among the group of smokers, a statistically significant difference existed only in men; but compared with the non-smokers, the correlation coefficient was smaller.

Ⅳ. Discussion

The salivary occult bleeding test is based on the following reactions: via the catalytic action of hemoglobin that is contained in the saliva, the organic peroxides oxidize ortho-triazine, developing a yellow-to-greenish-yellow coloration, depending on the hemoglobin concentration[11]. Based on this coloration, the extent of occult blood in the saliva can be determined. Gingival hemorrhage is one of the major symptoms of periodontal diseases: when an inflammation develops in the gingiva, occult blood is detected in the saliva due to hemorrhaging from the gingival crevices and pockets. The amount of occult blood increases as the inflammatory condition of the periodontal tissue progresses[12]. In the salivary occult bleeding test, the severity of the periodontal disease is determined by using this physiopathological feature.

The salivary occult bleeding test is useful for screening but hemorrhage from the periodontal tissue is affected by one's smoking habits. Several researchers reported that this test tends to overlook periodontal diseases among smokers. Thus far, from an epidemiological viewpoint, studies have been less than complete

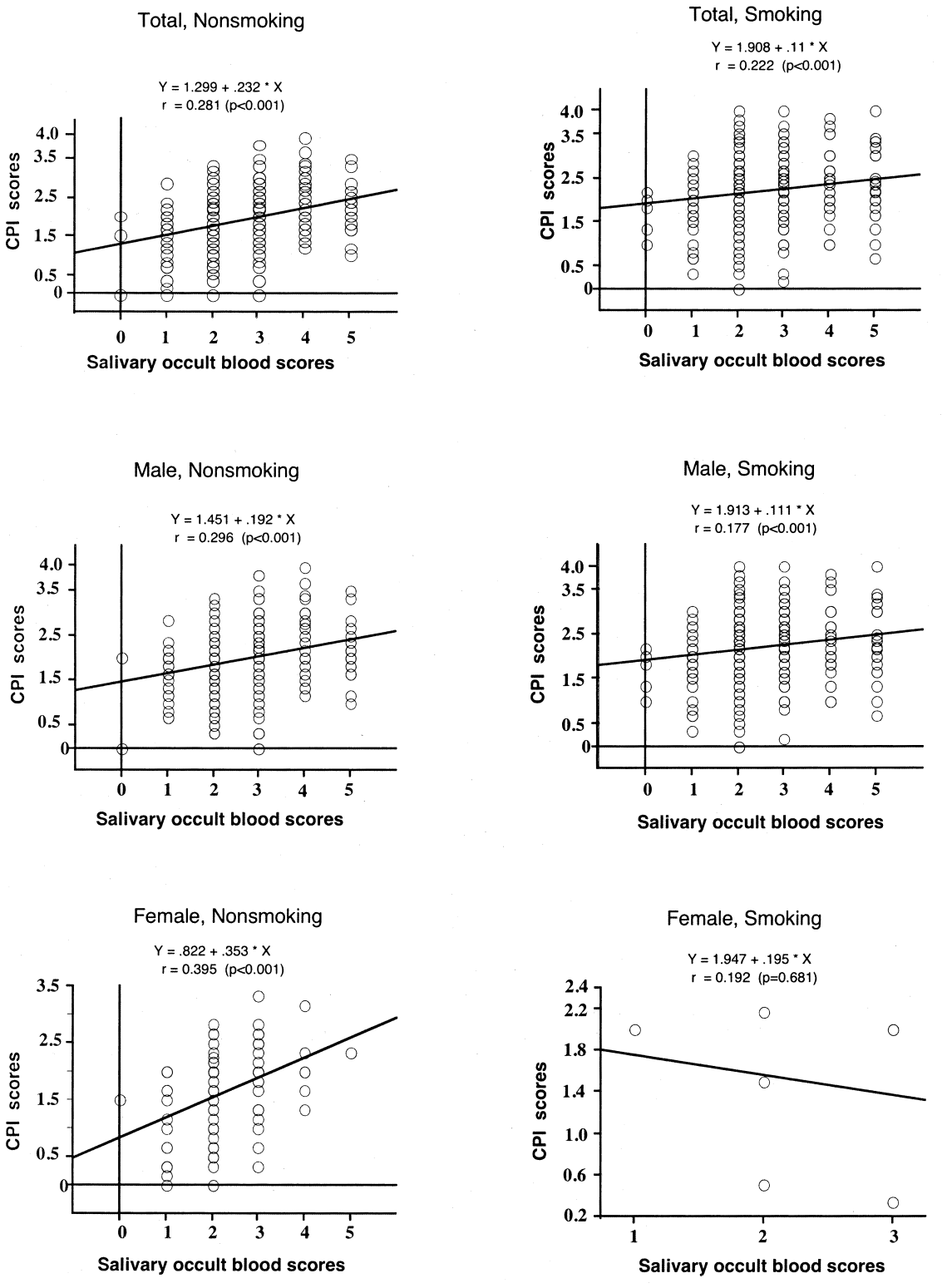

Fig. 3 The relationship between the CPI and the Salivary occult blood scores by smoking habits

on this point. The current study was undertaken on 1,616 employees of chemical plant to analyze the relationship between the result obtained from the salivary occult bleeding test and one's smoking habits.

Of the subjects of the current study, 55.7 % were men and 7.0 % women. Compared with the national statistics (Men; 48.3 %, Women; 13.6 %)[13], the proportion of men is very high in this group of subjects. Together with the salivary occult bleeding test, the CPI-in which the effect of smoking is limited-was determined and its correlation with smoking habits was investigated. The CPI is determined by dentists or dental hygienists by using a periodontal probe: the test is based on gingival bleeding following probing, the presence (or absence) of dental calculus, and the depth of a periodontal pocket. The method is relatively unaffected by smoking.

Progress has been made in epidemiological studies and those on the developmental mechanism by which periodontal diseases and smoking habits are related. In the past 5 years, more than 200 research reports have been published on these subjects[14]. In the United States, cohort studies were conducted on a large number of subjects, establishing that one's smoking habits have an effect on the development and progression of periodontal diseases. The mechanism by which smoking exerts its effects has also been elucidated, mainly in animal experiments. It is now well-accepted that nicotine that is inhaled through smoking causes vasoconstriction, the development of fibrous texture in the gingiva, and inhibition of leukocyte functions, all of which abet periodontal diseases to increase their severity. Thus the relationship between periodontal diseases and smoking habits has been proven from several perspectives[15].

The current study revealed that the following relationship exists between the CPI and one's smoking habits: the CPI is higher among smokers than non-smokers and it gains statistical significance in proportion to the increase in the amount of cigarettes smoked and the duration of smoking. When CPI is used as an index to determine the development of periodontal diseases, the odds ratio for having the disease appears to rise in proportion to the number of cigarettes smoked and the duration of smoking. In other words, it becomes evident that smoking contributes to an increase in the severity of the periodontal disease. The results of the current study, using CPI to evaluate periodontal diseases, conforms to those of many prior studies[15].

The relationship between the results obtained from the salivary occult bleeding test and one's smoking habits was also investigated in the current study. No significant correlation was found between the results of this test and smoking habits, number of cigarettes smoked, or duration of smoking. When the development of periodontal diseases was determined by using the results of the salivary occult bleeding test as an indicator, it was found that, unlike the study using the CPI, the odds ratio for periodontal diseases was inversely related to the number of cigarettes smoked and the duration of smoking. This was contrary to the results reported in general on the effects of smoking on periodontal tissue. When the correlation between CPI and the results of the salivary occult bleeding test was examined next, the correlation coefficient for the group of smokers was found to be smaller than that for the group of non-smokers. It is believed that smoking is the direct cause of this unexpectedly small correlation coefficient. Saliva does not appear to be an appropriate

medium for evaluating periodontal diseases in smokers. The effect of nicotine contained in tobacco smoke has been cited to explain this phenomenon. Nicotine stimulates the sympathetic nerves and causes the peripheral vasculature to contract[16]. In particular, it has been said that nicotine exerts a prominent effect on the peripheral vasculature of the skin and mucous membranes[17]. Thus it is readily conceivable that nicotine causes the blood vessels of the periodontal tissue to contract. Furthermore, the effect of nicotine is dependent on its blood concentration after it has been absorbed[18]; and it has been reported that circulatory disturbances (including shutting off the capillary blood flow) may develop, depending on how much one smokes. Therefore it is understood that a circulatory dysfunction develops; and the extent of hemorrhage is reduced in proportion to the increase in the number of cigarettes smoked and the duration of smoking[19]. In other words, it is believed that the salivary occult bleeding test, which uses occult blood derived from the gingiva as an index, may cause false-negative reactions among smokers because hemorrhage, the marker for the test, is suppressed with increases in the number of cigarettes smoked and the duration of smoking. Thus one may assume that the saliva test overlooks periodontal diseases in smokers.

As described above, the salivary occult bleeding test -as a screening test for periodontal diseases-is simple and objective; and unlike other tests, it obviates the need for services by dentists and other professionals. Therefore it is effective and useful as a screening test. However, the incidence of false negative reactions is high in individuals who smoke many cigarettes or have been smoking for a long time. This test should be conducted only among non-smokers.

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要約

唾液潜血反応試験による歯周疾患の評価において、喫煙者は歯周疾患の偽陰性率が高いとされている。我が国の男性の喫煙率が約50 %であることを考慮すると、唾液潜血反応試験と喫煙習慣の関連性について精度の高い疫学研究が必要である。しかし、我が国では対象者数、選択バイアスの可能性など、調査方法に問題があると考えられるものが多く、十分な疫学的知見が得られていない。このため、本研究では大規模事業所従業員1,616 名を対象に、唾液潜血反応試験に及ぼす喫煙習慣の影響を CPI を使用した歯周疾患の評価と比較して検討した。

その結果、CPI で評価した場合、歯周疾患に罹患するオッズ比は非喫煙者、現在非喫煙者、喫煙者の順に高く、また、喫煙本数、喫煙期間の増加につれて高くなった。唾液潜血反応試験により評価した時は、CPI とは反対の傾向を示した。歯周疾患罹患における唾液潜血反応試験と CPI の相関をみた結果、非喫煙者においては男女とも有意な相関関係が認められたが、喫煙者においては男性のみに有意な相関が認められた。しかし、その差異は非喫煙者に比べて小さかった。

以上の結果から、唾液潜血反応試験は集団を対象とした歯周疾患のスクリーニングテストとしては有効、有用である。しかし、唾液潜血反応試験は、喫煙者、特に喫煙量、喫煙期間の多い者については偽陰性率が高くなるため、非喫煙者のみを対象とすべきであることが認められた。

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