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Measurement of Carbon Monoxide in Exhaled Breath as a Possible Marker of Stress

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We measured the concentration of carbon monoxide (CO) gas in exhaled breath and the level of stress in students to evaluate their lifestyle and mental health for the annual medical checkup at our University. 993 students were examined for CO by gas analysis device and 445 selected randomly from them for stress levels by a Stressometer. CO concentration in breath is markedly higher in the case of smokers and slightly higher in non-smokers who are exposed to passive smoking. Students with a tremor of the nervous system at rest (TNR) higher than 70 in a Stressometer were found in 13.0%, and in nonsmoking female students there was a significant correlation between stress levels and CO concentration in the breath which is produced in the cells by stress-induced heme oxygenase-1. The results of the present study suggest that measuring CO gas may be a convenient way to evaluate lifestyle and stress levels in students.

Key words —— breath carbon monoxide, stress meter, heme oxygenase-1, stress, smoking

INTRODUCTION

There has been much talk recently of the influence of different stressors in daily life on the heart and on the body, and excessive anxiety or tension caused by psychological stress risks induces abnormal secretion of stress hormones which could consequently develop into autonomic nerve disorders.[1) These body responses provoke various mental illnesses such as depression and/or so-called lifestyle-related diseases such as hypertension and diabetes.[2) Development of low invasive methods for clinical testing in order to obtain information on the living body is anticipated in the future. One of these methods may well involve breath gas. The Japanese Society of Clinical Biochemistry on Biogas Analysis was founded in 1998 to advance this new field of clinical medicine and the International Association for Breath Research was then founded in 2002 with the aim of using breath gas for purposes of clinical diagnosis and therapeutic monitoring. The members of both associations are involved in developing a new machine for sensitively measuring small quantities of breath gas which may be useful for clinical diagnosis and therapy. Taking advantage of the opportunity presented by the annual medical check-up on students at the Kawasaki University of Medical Welfare, where we specialize in the field of training of experts in medical care, we measured the concentration in the breath of carbon monoxide (CO) produced in the cells by heme oxygenase-1 (HO-1),[3) which is induced by oxidative stress, as well as levels of psychological stress using a Stressometer device (described as a stress meter in the following text).[4,5) Recent studies[6,7) have suggested that psychological stress induces the production of reactive oxygen species, which can result in oxidative stress and inflammatory reaction. We thought that measuring these indicators simultaneously would make it possible for us to evaluate the levels of psychological stress experienced by students and their lifestyle, as manifested in their smoking habits (considered as exogenous CO)[8) and physical activity. This evaluation is likely to be useful for orienting students in our university towards the medical career on which they will embark in the future. In effect, the analysis of gas in the breath is non-invasive and adequate for repeated analysis.[9) In addition, a new gas measurement device makes it possible to analyse CO.[10) Two of the authors (A.W. and H.U.), both of whom are on the staff of the Japanese Society of Clinical Biochemistry on Biogas Analysis, succeeded in developing this machine for use in investigating people's lifestyles and possibly their mental stress levels as well. Moreover, we have been fortunate in having had the opportunity to use a stress meter (Stressometer) to measure quanti-

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tatively the level of psychological stress which was recently developed by Comby *et al.*[4)]

For all these reasons, we planned this experiment at the time of the students' medical check-up in order to acquire knowledge of their levels of stress and lifestyle. Although the present study would appear to be the first report on the results of CO gas in the breath and stress levels using a stress meter, fundamental analysis is needed to interpret the obtained data. The present paper shows a summary of the results of this trial.

MATERIALS AND METHODS

Medical Check-Up of Students —— The annual medical check-up of students at our university (three faculties consisting of twelve departments and with 4000 students) took place between 28 March and 13 April 2005. During this period and depending on our availability, we took samples for measuring CO gas in the exhaled breath of 993 students. At the same time, we also measured the stress levels of 445 students, who were randomly selected from the above 993 students, using a stress meter. Before measurement of exhalation and stress levels, the students filled out a questionnaire which was used for self-evaluation at the time of the analysis. Informed consent was obtained from all students after we had thoroughly explained the objectives, methods and other relevant details of the study. This research has been approved by the Ethics Committee of our university under the Approval Number 018.

Exhaling Sampling —— Each student breathed in lightly and then waited 15 sec before exhaling. A breath sample was taken at the last moment of exhalation, with each subject exhaling between 100 and 200 ml into a breath-sampling bag manufactured by Otsuka Pharmaceutical Co., Ltd. (Osaka, Japan).

Measurement of Exhaled Gas —— One ml of exhaled gas was taken with a syringe and introduced into a gas analysis device, TRIlyzer mBA-3000, manufactured by Taiyo, Co., Ltd. (Osaka, Japan), which makes it possible to analyse CO gas for 5 min per sample. All the analyses were performed two to five days after the exhalation was sampled. The analysis device used is a gas chromatograph equipped with a high-sensitivity semiconductor gas detector. The lower limit for detection of the CO gas is 0.1 ppm, and its reproductivity is ±2%. Its linearity reaches a level of 100 ppm.[10)] We used pure synthetic air (impurity of less than 0.1 ppm) as the carrier gas. We carried out calibration on every 100 samples using mixed gas of high concentration (50 ppm for the three gases) and low concentration (5 ppm). The concentration of CO in the air where the analysis was performed was 0.6 ppm.

Stress Level —— The stress level was measured using a stress meter (Stressometer) developed by TRN Technologies (Paris, France).[4)] This responds to a tremor (the amplitude of microvibration) in the rhythm range of 3 to 20 Hz. While the measurement was being made the students maintained the standard position for measurement (standing but relaxing). They lightly grasped the sensor handle with the right hand (or the left hand in the case of left-handed subjects) and waited 10 to 20 seconds for the TNR (tremor of the nervous system at rest) to stabilise at a level below 50 TNR in the "Continuous" mode.[4, 5)] We then measured stress for 20 sec in the "Measurement" mode.[4, 5)] If the value obtained was in excess of 100 TNR, we carried out the measurement again after asking the student to assume a relaxed posture. The stress meter makes it possible to detect tremor of the hand with a rhythm range between 3 and 20 Hz (times per seconds) by means of a piezo-electric sensor. The electronic circuit processes the signal and displays the measured TNR value on the screen. In most cases the average TNR value is situated between around 25 and 50 in the case of a healthy man.

Statistical Analysis —— The results obtained are expressed in terms of the mean value ± the standard deviation, and the significant difference is verified by means of Student *t*-test. We estimated the significant difference when p is less than 0.05.

RESULTS

CO in the Breath

The concentration of CO in the breath is classified into the following three categories: Category 1: under 3 ppm, Category 2: 3 to 5 ppm, Category 3: over 5 ppm. Table 1 shows the results. Among the 993 students who took part in the test, 11.3% came within Category 3 (over 5 ppm). The figure for male students only (n = 382) indicated that 20.2% came within Category 3, while the figure for female students (n = 611) shows that only 5.7% came within this category. This difference is significant ($p < 0.01$) (Table 1 and Fig. 1). Figure 2 shows the number of smokers and non-smokers as well as exposure to passive smoking by non-smokers, with

Table 1. Results of Measurement of Concentration of CO Gas in the Exhaled Breath and of Measurement of Stress Levels among Students (According to Gender, in %)

Designation	Category	Total Number (%)	Male (%)	Female (%)
Concentration of CO in Exhaled Breath (ppm)	Under 3	494 (49.7)	148 (38.7)	346 (56.6)
	3 to 5	378 (41.1)	157 (41.1)	230 (37.7)
	Over 5	112 (11.3)	77 (20.2)	35 (5.7)
	Total (%)	993 (100)	382 (100)	611 (100)
Stress Level (TNR)	Under 25	61 (13.7)	23 (13.2)	38 (14.0)
	25 to 49	245 (55.1)	82 (47.1)	163 (60.2)
	50 to 69	81 (18.2)	36 (20.7)	45 (16.6)
	70 to 100	45 (10.1)	23 (13.2)	22 (8.1)
	Over 100	13 (2.9)	10 (5.8)	3 (1.1)
	Total (%)	445 (100)	174 (100)	271 (100)

We measured the stress levels of 445 students, who were randomly selected, among 993 students being measured for CO gas in the breath.

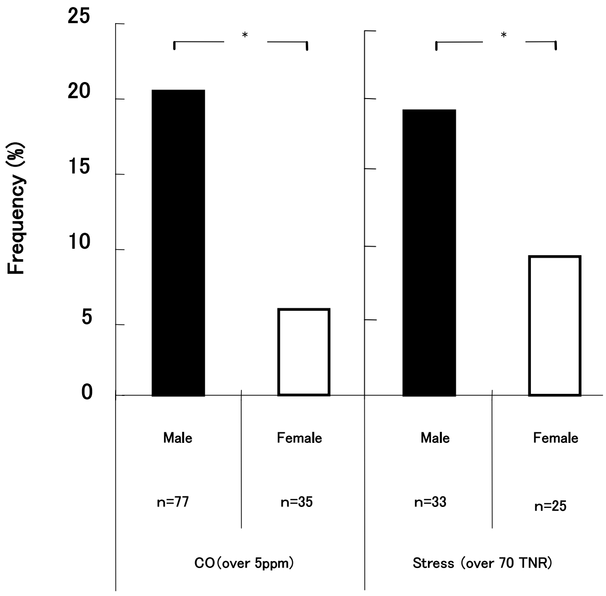

Fig. 1. Comparison of High Values for Concentration of CO in Exhaled Breath and Stress Levels and Differences Therein According to Gender
$^*p < 0.01$.

a classification of the risk of passive smoking into the following three categories: Perhaps risk; Maybe no risk; No risk. CO concentration is markedly higher in the case of smokers, and the concentration increases in proportion to the number of cigarettes smoked.[8] In the case of non-smokers, CO concentration is higher in the case of those who are exposed to passive smoking than in the case of non-smokers who are not exposed to this risk ($p < 0.01$) (Fig. 2). These results show that the difference due to gender on the concentration of CO in the breath is likely to be due to the difference in the number of smokers, because of the greater proportion of smokers among male students (79.3%). Among non-smokers who are not exposed to passive smoking, we also carried out a comparison of the concentration of CO involving division into the two following groups: Those who engage in physical activity (two or three times a week and four or five times a week) and those who do not engage in any physical activity. The concentration of CO is higher in the group engaging in physical activity (two or three times a week and four or five times a week) than for the group not engaging in physical activity. The concentration of CO increases in accordance with the frequency with which physical activities are engaged in, and the difference in this respect is significant ($p < 0.01$). We also performed a comparison of the concentration of CO in the breath in accordance with the replies "Yes" or "No" given by students in the questionnaire (energetic physical activity in the preceding day, alcoholic beverages, regular meals, constipation, duration of sleep, taking of medicines or dietary supplements, fatigue or stress, unstable health), but we failed to find any significant differences.

Level of Stress According to Level of Hand Tremor

We measured the level of psychological stress of students using a stress meter and classified the results according to gender into the following five categories: Under TNR25, TNR25 to TNR49. TNR50 to TNR69, TNR70 to TNR100, over TNR100. The TNR for most students (68.8%) was less than 50. But 13.0% of students had a TNR higher than 70. Between men and women, a TNR higher than 70 is more important for a man than it is for a woman ($p < 0.01$) (Table 1 and Fig. 1). We also studied the

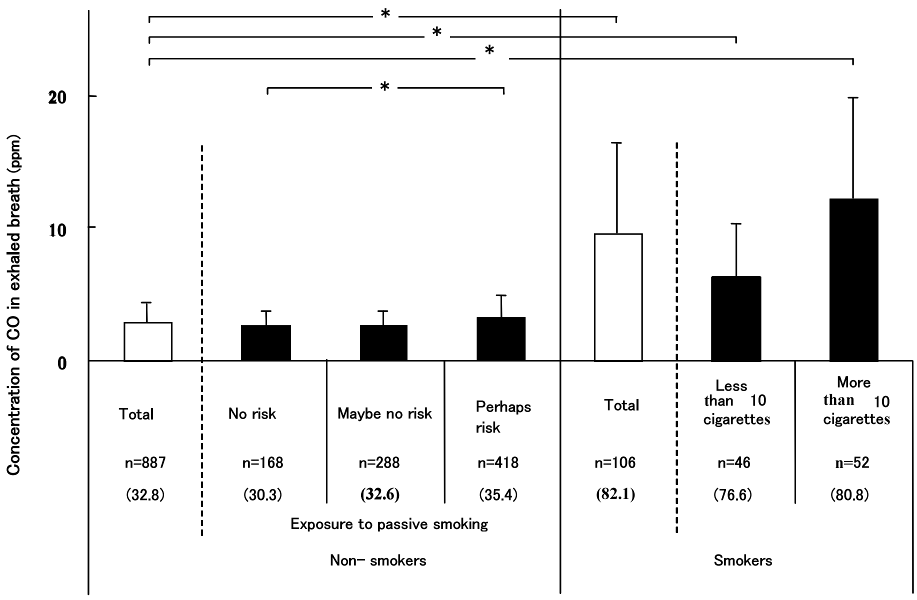

Fig. 2. Comparison of Concentration of CO in Exhaled Breath between Smokers and Non-smokers (Classified in Accordance with Exposure to Passive Smoking)

Eight students among 106 smokers did not reply concerning the number of cigarettes smoked and 13 students did not state whether they were exposed to passive smoking in the Questionnaire. Figures in brackets show the percentage of male students. Concentration of CO over 5 ppm was observed in 11 of 168 students who were not exposed to passive smoking. The numbers of students smoking 20 to 30 cigarettes and more than 30 cigarettes were 2 and 1, and the concentrations (average) of CO were 19.0 and 24.3 ppm, respectively. $^{*}p < 0.01$.

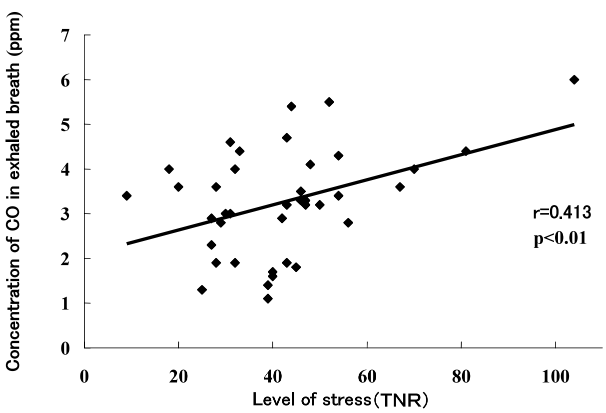

Fig. 3. Correlation between Stress Levels and Concentration of CO in Exhaled Breath for Non-smoking Female Students Not Exposed to Passive Smoking

$y = 0.0281X + 2.07$, $R^2 = 0.1706$ ($p < 0.01$). The number of female students were 36 selected randomly from 445 students who were simultaneously measured for CO gas using a gas analysis device and stress levels using a stress meter.

correlation between stress levels and the concentration of CO in the breath (considered as a stress marker) for the case including smokers and passive smokers as well as for the case excluding smokers and passive smokers, but we did not manage to find any clear correlation between these two cases. On the other hand, there was a significant correlation for non-smoking female students (n = 36, excluding passive smokers; r = 0.413, $p < 0.01$) (Fig. 3). There was no clear correlation with replies to the questionnaire (smoking habits, daily physical activity, energetic physical activity, regular meals, constipation, length of sleep), nor did we find any correlation as regards replies concerning fatigue or stress.

DISCUSSION

Results obtained by a gas analysis device developed by us and a stress meter interestingly showed that the proportion of students with a TNR higher than 70 in a stress meter was 13.0%, and in non-smoking female students there was a significant correlation between stress levels and CO concentration in the breath. Since CO is produced in the body by an enzymatic reaction of HO-1 induced by oxidative stress, one can consider CO to be a powerful anti-stress element. Accordingly, a series of reactions induced by HO-1 should provide a biological defence.[11] A number of non-smokers also showed a high level of CO in their breath. It seems likely that this phenomenon is due to passive smoking or to the inhalation of exhaust gas from road vehicles.[12] However, among non-smoking students not exposed to passive smoking, we noticed a CO concentration in excess of 5 ppm in 10 out of 168 students, and this might be connected, for example, to physical activity. It should be observed too that there is a correlation between the level of stress measured by a stress meter and the concentration of CO for non-smoking female students with no exposure to passive smoking. In order to clarify the influence of stress on illness, we will need to re-check the variation of CO concentration during the day as well as before and after the stress charge.

A stress meter can measure minute tremors (responding to tremor in the rhythm range of 3 to 20 Hz) in the hand during rest. Physiological tremor or essential tremor is the most frequently observed type of tremor (8 to 13 Hz) even in healthy people. Neither type of tremor is evident while at rest and they are thus classified into tremor while in motion and posture (kinetic/action tremor). Kinetic/action tremor is therefore as far as possible excluded by a stress meter, and thus tremor arising due to stress is mainly measured. A number of reports have been published on how mental stress has the effect of increasing the amplitude of tremor.[13–15] However, more efforts to distinguish changes attributable to stress from some types of physiological tremor (assuming that stress is not involved) should be carried out.

Further studies are also needed to determine whether or not the data obtained here will be useful for evaluating people's lifestyles and psychological and/or oxidative stress levels, for example, at the time of the annual medical check-up. The mutual relationship between the CO gas measured and lifestyle or stress will be examined in detail in the future.

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