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*Original Article*

# Serum Thiocyanate Concentration as an Indicator of Smoking in Relation to Deaths from Cancer

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## Abstract

**All residents aged 40 years or more in Oyabe City, Toyama Prefecture, Japan were involved in an annual medical check-up between 1987 and 1988. The cohort was followed and death certificates from cancers were confirmed prospectively. During follow-up to December 31st, 1994, 100 deaths (28 gastric, 17 lung and 55 other cancers) from cancers occurred, and these subjects were included in this study as the case group. Subjects in the control group, matched for gender and age with the cases, were selected randomly from participants whose serum samples had been stocked during annual medical check-up. The concentration of serum thiocyanate in all (79.8 μmol/l), gastric (86.7 μmol/l) and lung (90.0 μmol/l) cancer patients were significantly higher than that of relevant controls (64.3 μmol/l, 59.0 μmol/l and 61.0 μmol/l, respectively; and $p<0.001$, $p<0.001$ and $p<0.05$, respectively). After adjusting for BMI, blood pressure and total serum cholesterol, the results of multiple logistic regression analysis showed that the risk of all cancers (OR=3.40, 95% confidence interval (95% CI): 1.67–6.96, $p<0.01$), gastric cancer (OR=7.98, 95% CI: 1.91–33.34, $p<0.05$) and lung cancer (OR=8.83, 95% CI: 1.19–65.65, $p<0.05$) were elevated significantly with logarithm transformed values of serum thiocyanate increased. The present findings suggested that in epidemiological studies confirmation of smoking status with biomarkers such as serum thiocyanate may be important, although considering the small sample size, a relatively weaker risk to interested factors rather than the strong relationship between smoking and cancer was noted.**

**Key words**: serum thiocyanate, lung cancer, gastric cancer, smoking

## Introduction

Self-reported information on smoking status, obtained by interview or a questionnaire, is commonly used to estimate the relationship between smoking and the risk of certain cancers. Although it can provide much information on smoking status, the reliability of such findings is limited[1)]. It is often that the self-reported information cannot reflect the differences in the rate and depth of inhalation between smokers or the types of cigarette smoked, both of which may affect the amount of harmful compounds inhaled[2)]. Furthermore, it relies totally on truthful reporting by the subjects and with many smokers denying their habit, a more objective and accurate quantitative approach to the extent of exposure to tobacco smoking is often necessary[3)].

There are several biological markers that could be used to verify self-reported information[4)]. Expired air carbon monoxide (CO), carboxyhemoglobin (COHb), plasma cotinine, serum thiocyanate, and plasma and urinary nicotine are measures used to determine smoking exposure[1)]. Hydrogen cyanide is found in the gases of tobacco smoke. In the human body, hydrogen cyanide is metabolized to thiocyanate[5)]. The relatively long half-life (about 1–2 weeks) of serum thiocyanate makes its measurement particularly suitable for evaluating average smoking exposure. It might be expected to identify persons who stopped smoking only for the day of their clinical appointment. The validity of serum thiocyanate as an indicator of cigarette smoking has been previously studied[1)].

However, there was few epidemiological investigation on the relationship between serum thiocyanate level and cancer morbidity or mortality directly[6)]. We investigated the serum thiocyanate concentration and cigarette smoking in relation to deaths from

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cancer using a nested case-control design in a community-based cohort study.

## Subjects and Method

*Participants, design, and measurement*

All residents aged 40 years or above (6,954 persons, including 2,421 males and 4,533 females) in Oyabe City, Toyama Prefecture, Japan were involved in an annual medical check-up between 1987 and 1988. The cohort was followed and death certificates from cancers were confirmed prospectively. During the follow-up period until December 31st, 1994, 100 deaths from various cancers had occurred, and were included in this study as the case group. The diagnosis of cancer was made with reference to the reporting physician's diagnosis and other relevant information following the ninth revision of the International Classification of Diseases. The subjects were 54 males and 46 females, and the average age was 68±9 years. Among the cancers, there were 28 stomach, 17 lung and 55 other types of cancers.

Subjects in the control group (100 persons) were matched for gender and age with the cases, and were selected randomly from the cohort whose serum samples had been stocked in an annual medical check-up between 1987 and 1988. Information about age and self-reported status of cigarette smoking was obtained from questionnaires. The height, weight, blood pressure, and concentration of total serum cholesterol was taken from their annual medical check-ups for both case and control subjects. According to the self-reported smoking habits each subject was classified into subgroups of non-smokers, light smokers who smoke 10 cigarettes or less per day, moderate smokers who smoked 11–20 cigarettes per day, and heavy smokers with more than 20 cigarettes per day. The body mass index (BMI) (weight in kg per square of height in meters, $kg/m^2$) and mean blood pressure (MBP) were also calculated.

Serum thiocyanate was measured by the modified method of Butts et al[7]. using frozen serum samples, which were collected at the annual medical check-up between 1987 and 1988. A volume of 0.8–1.0 ml of serum samples was diluted to 2 ml with 1 ml of $NaClO_4$ (0.1 M) added and shaken, then 1 ml of trichloroacetic acid (30% W/V) was added and shaken vigorously. It was stored for 20 minutes at room temperature, and subsequently centrifuged for removal protein-precipitates. One milliliters of ferric nitrate $[Fe(NO_3)_3 \cdot 9H_2O]$ (10 g/l) was added to 2 ml of clear supernatant, and shaken vigorously. Thereafter, absorbance at 455 nm of the samples was measured immediately. The quantitative of thiocyanate was done along with standard curve made by taking KSCN (50–200 μmol/l solution) as the standard of $SCN^-$ running through the same operation as used for the serum samples. The method has been accepted and validated previously[8].

*Statistical methods*

Between the case and control groups, the differences in the mean values of age, height, weight, BMI, DBP, SBP, BMP, and serum total cholesterol were examined by the paired t-test. The distribution of the serum thiocyanate concentration did not follow a normal distribution. Thereforen, the values of serum thiocyanate were naturally logarithmically transformed to produce normal distribution. The ratio of smokers to non-smokers and the ratio of males to females were examined by the chi-squared test. The multiple logistic regression analysis was used to examine the effect of serum thiocyanate and other factors on the cancer mortality, with the results shown as odds ratio (95% confidence interval). The sample size of this study was not large enough to permit further analysis by stratifying some factors such as gender and age groups. All statistical analyses were performed using the SAS 6.12[9,10] software package.

## Results

The general characteristics of those participating in the study are shown in Table 1. The proportion of gastric cancer to total cancers in this population was 28%, which was higher than the 22.1% for the population of Toyama Prefecture and 23.4% for the national population. However, the proportion of lung cancers was 17%, which was similar to the population of the Prefecture (17.2%) and Japan (16.2%)[11]. There was no significant difference

**Table1 General Characteristics of cancer patients and controls (paired t-test)**

| | All cancers | | | | Stomach cancer | | | | Lung cancer | | | | Other cancers | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Controls (100) | | Patients (100) | | Controls (28) | | Patients (28) | | Controls (17) | | Patients (17) | | Controls (55) | | Patients (55) | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Age (years) | 67.6 | 8.5 | 67.6 | 8.5 | 67.9 | 8.8 | 67.9 | 8.9 | 69.1 | 6.6 | 69.1 | 6.6 | 67.0 | 8.9 | 67.0 | 8.9 |
| Height (cm) | 153.7 | 8.4 | 151.2 | 9.3 | 154.7 | 8.4 | 152.4 | 9.0 | 156.7 | 6.3 | 154.4 | 8.7 | 152.2 | 8.7 | 149.6 | 9.4 |
| Weight (kg) | 54.0 | 9.0 | 51.0 | 8.2 | 54.1 | 10.1 | 52.3 | 8.1 | 56.9 | 8.8 | 49.4 | 8.2 | 53.0 | 8.5 | 50.9 | 8.2 |
| BMI ($kg/m^2$) | 22.8 | 3.0 | 22.3 | 3.0 | 22.5 | 3.2 | 22.5 | 2.6 | 23.1 | 2.9 | 20.7 | 3.0 | 22.9 | 2.9 | 22.7 | 3.1 |
| DBP (mmHg) | 76.9 | 10.4 | 76.8 | 1.4 | 78.5 | 11.6 | 80.4 | 10.5 | 79.4 | 10.7 | 77.6 | 12.0 | 75.3 | 9.6 | 74.7 | 11.3 |
| SBP (mmHg) | 131.9 | 18.3 | 134.0 | 20.3 | 133.3 | 19.3 | 138.6 | 21.9 | 140.7 | 16.2 | 133.4 | 20.0 | 128.4 | 17.6 | 131.8 | 19.4 |
| MBP (mmHg) | 95.2 | 11.8 | 95.8 | 13.1 | 96.8 | 13.1 | 99.8 | 13.3 | 99.8 | 10.8 | 96.2 | 14.0 | 93.0 | 11.0 | 93.7 | 12.5 |
| T-chol (mg/dl) | 199.6 | 40.1 | 197.3 | 39.4 | 196.1 | 30.7 | 191.3 | 37.2 | 194.8 | 40.6 | 186.1 | 42.3 | 203.4 | 44.9 | 205.1 | 38.8 |
| Thiocyanate[a] (μmol/l) | 64.3 | 1.4 | 79.8*** | 1.6 | 59.0 | 1.4 | 86.7** | 1.6 | 61.0 | 1.4 | 90.0** | 1.6 | 68.3 | 1.5 | 73.7 | 1.5 |
| Gender Male(%)[b] | 54.0 | | 54.0 | | 60.7 | | 60.7 | | 76.5 | | 76.5 | | 43.7 | | 43.7 | |
| Smoking (%) [b] | 22.0 | | 36.0 | | 14.3 | | 42.8 | | 23.5 | | 58.8 | | 25.4 | | 25.4 | |

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; MBP, mean blood pressure; T-chol, serum total cholesterol.

[a] The paired t-test was conducted between cases and controls with logarithmically transformed values of serum thiocyanate, then the results were changed back;

[b] chi-squared test.

*** P<0.001 ** P<0.01 patients vs controls.

**Table 2 Analysis of the risk of cancer using multiple logistic regression analysis (Odds ratio (95% confidence interval))**

| | All cancers | Stomach cancer | Lung cancer | Other cancers |
|---|---|---|---|---|
| BMI | 0.96 (0.50–1.84) | 0.96 (0.24–3.88) | 0.41 (0.05–3.16) | 1.07 (0.46–2.47) |
| MBP | 1.09 (0.41–2.91) | 1.02 (0.18–5.96) | 0.57 (0.09–3.81) | 2.91 (0.29–29.2) |
| T-Chol | 0.81 (0.44–1.50) | 0.83 (0.27–2.60) | 1.40 (0.32–6.11) | 0.67 (0.29–1.56) |
| Thiocyanate | 3.40 (1.67–6.96)** | 7.98 (1.91–33.34)* | 8.83 (1.19–65.65)* | 1.55 (0.60–3.99) |

** $p<0.01$ * $p<0.05$.
BMI, body mass index; BMI>=24 vs BMI<24 kg/m$^2$.
MBP, mean blood pressure; BMP>=110 vs BMP<110 mmHg.
T-Chol, serum total cholesterol; T-Chol>=200 vs T-Chol<200 mg/dl.
Thiocyanate, natural logarithm transformed values of serum thiocyanate.

between cancer patients and controls for mean age, height, weight, BMI, blood pressure and serum total cholesterol. The proportions of self-reported smoking were higher among all cancer patients (36%), stomach (42.8%) and lung (58.8%) cancer patients than those of relevant controls (22.0%, 14.3%, and 23.5%, respectively).

As one of the biological markers of smoking status, the concentration of serum thiocyanate in all (79.8 μmol/l), gastric (86.7 μmol/l) and lung (90.0 μmol/l) cancer patients were significantly higher than that of relevant controls (64.3 μmol/l, 59.0 μmol/l and 61.0 μmol/l, respectively) ($p<0.001$, $p<0.001$ and $p<0.05$, respectively). The concentration of thiocyanate in patients with other types of cancer (73.7 μmol/l) was also higher than that of controls (68.3 μmol/l), but not significantly.

The results of multiple logistic regression analysis, analyzing the risks of cancers, are shown in Table 2. The age and gender has been matched between patients and controls. After adjusting for other general factors such as BMI, blood pressure and total serum cholesterol, the risk of all cancers (the odds ratio was 3.40 with the 95% confidence interval (95% CI) of 1.67 to 6.96, $p<0.05$), gastric cancer (7.98 with 95% CI of 1.91 to 33.34, $p<0.01$) and lung cancer (8.83 with 95% CI of 1.19 to 65.65, $p<0.05$) elevated significantly with increments in the logarithmically transformed concentrations of serum thiocyanate. The risk of other types of cancers was not significantly changed with higher concentrations of serum thiocyanate. When the self-reported status of smoking was included in the multiple logistic regression, instead of the concentration of serum thiocyanate, the only significantly elevated risk was for the lung cancer ($p=0.05$). (detailed data not shown).

As expected, the average concentration of serum thiocyanate was elevated significantly with increasing numbers of cigarettes smoked per day, although the correlation coefficient between the two variables was not very large ($r=0.414$, $p<0.001$). The concentration in non-smokers was 69.9±29.3 μmol/l, which was significantly lower than that of light (92.2±39.9 μmol/l), moderate (106.7±72.0 μmol/l) and heavy (164.0±41.6 μmol/l) smokers.

## Discussion

In epidemiological studies on cigarette smoking, self-assessment reports and direct interviews have usually been taken as an indicator of smoking status. However, misclassification due to these methods is inevitable, because, for example, individuals sometimes mistake their cigarette consumption and smokers who have failed and/or want to stop smoking are apt to under-report. Patients may often say what they think the doctor wants to hear, rather than admit to not doing as advised. The use of serum thiocyanate levels confirmed a higher level of smoking denial by smokers in one disease (ischaemic heart disease) than in another disease (peripheral vascular disease)[3]. Moreover, self-reporting of smoking has shown a marked degree of digit preference, with the vast majority of smokers reporting in multiples of 10 cigarettes per day. Therefore, self-reports of the number of cigarettes per day may be biased towards round numbers, rather than the exact number of cigarettes smoked per day[12]. In addition to actual cigarette consumption, smoking status varies with depth of inhalation, frequency of puffing and the proportion of each cigarette consumed. The amount of tobacco products in the smoke from a cigarette is higher from the last part of the cigarette than from the first part, and is also higher when the cigarette is smoked rapidly and inhaled more strongly than when it is smoked slowly[13]. Therefore, it is important that we make an effort to gain an objective marker of smoking status.

To assess smoking status objectively, carbon monoxide in expired air, blood carboxyhemoglobin (COHb), serum cotinine and serum thiocyanate have been measured[14]. Among these indicators, expired carbon monoxide is easily measured and blood carboxyhemoglobin measurements have been substituted by expired air carbon monoxide measurements. However, carboxyhemoglobin has a relatively short half-life of about four hours and, therefore, its concentration in the blood remains abnormal only for a short time after cessation of smoking. It is likely to be the least accurate, and may also be raised by environmental exposure to products of incomplete combustion[15]. Cotinine, a metabolite of nicotine, is specific to cigarette smoking and is one of the best indicators to distinguish smokers from non-smokers, but it also has a short half-life (30 hours), and its measurement requires a complex and expensive gas-liquid chromatographic technique.

Serum thiocyanate is a metabolite of hydrogen cyanide in tobacco smoke and has a relatively long half-life (about 1–2 weeks)[16]. Serum thiocyanate has the advantage that it better reflects the average exposure to tobacco smoke during the previous weeks, rather than exposure during the previous day. Therefore, thiocyanate is suitable for evaluating smoking habits, although it is not specific to tobacco smoking. Serum levels of thiocyanate have been reported by several investigators. Foss et al.[17] reported that the mean serum thiocyanate level in non-smokers was 33.9 μmol/l for males and 33.5 μmol/l for females. In moderate smokers (15–19 cigarettes per day), the mean level was 76.3 μmol/l for males, and in heavy smokers (more than 25 cigarettes per day), the mean level was 87.4 μmol/l for males. In a study on a Japanese rural population, Kasagi et al. reported that the optimal cut-off point, selected to differentiate smokers from non-smokers, was 50 μmol/l. Levels>70 μmol/l haves also been accepted as indicating smoking in other studies[18]. However, the

present findings shows that the serum concentration of thiocyanate in non-smokers is a little higher than the upper value in non-smokers. Thiocyanates and other cyanagens can be found in some kinds of plant foods such as cabbage, broccoli, cauliflower, garlic, brussels sprouts, horseradish, etc., and some are the typical vegetables consumed in the area of the study subjects. Diets which are particularly high in these food stuffs could thus produce higher serum thiocyanate levels. Thiocyanate levels can also rise during and immediately after smoking one single cigarette[14]. In this study, the smoking status was gathered by self-reporting the number of cigarettes smoked, therefore, the average concentration of serum thiocyanate in these groups depended heavily on the accuracy of the self-reported response.

The main objective of this study was not to explore the causal relationship between smoking and lung or other cancers, which has been examined and established by laboratorial and epidemiological studies during the past decades. However, the present findings suggested that in epidemiological studies confirmation of smoking status with biomarkers such as serum thiocyanate may be important, considering the small sample size, and the relatively weaker risk to interested factors rather than the strong relationship between smoking and cancer. Smoking is often one of the most important confounding factors in many epidemiological studies. Biomarkers like serum thiocyanate may be used as a more accurate index of smoking status than the self-reported number of cigarettes smoked, therefore, it can be used more effectively to control confounding effects of smoking. In a nested case-control study, serum samples can be stored at low temperatures for several years for measuring the concentration of serum thiocyanate.

The test of serum thiocyanate is highly dependent on the operator and the higher concentrations of serum thiocyanate can be the result of dietary factors[19]. As a design of a nested cohort study, we could not collect the information about the diet habit of subjects, which had not been included in the cohort study at the beginning, and this may have some negative effects on our findings. In addition, to small sample size, we also could not control the effects of some confounding factors such as occupational status, family history of diseases, life-style, and others. Even though there were several limitations in the present study, we observed a more significant relationship between serum thiocyanate and malignancies than between self-reported smoking status and cancer. The findings of the present study also showed, consistent with previous observations, a linear relation between the number of cigarettes smoked per day and the concentration of serum thiocyanate, although the correlation coefficient was not very large.

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