Smoking and use of smokeless tobacco in treated hypertensive men at high coronary risk: utility of urinary cotinine determination

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Accepted: 20 March 2002

ABSTRACT

Guidelines for the treatment of hypertension underline the central importance of strenuous efforts to reduce the prevalence of smoking, as epidemiological studies consistently have demonstrated that smoking increases the risk of cardiovascular disease and death by some two- or three-fold. Accuracy of a questionnaire is examined against the ability of urinary cotinine determination to distinguish between men exposed to tobacco (94 smokers [25 %], 30 snuff users [8 %]) and men not exposed (n=257), all of whom where treated hypertensives and were associated with at least one of the following factors: smoking, diabetes mellitus, serum cholesterol ≥ 6.5 mmol/L. Main outcome variables in this cross-sectional study of 381 men were cotinine concentration and cotinine:creatinine ratio in overnight urine samples (decision limits: 2 μmol/L and 1.0 mmol/mol, respectively); tobacco use according to questionnaire; and follow-up examination by questionnaire of alleged non-smokers with high urinary cotinine levels. Questionnaire sensitivity was 85%, whereas the urinary cotinine assay showed 98% sensitivity and 99% specificity. Fourteen (15%) out of 94 patients may have used tobacco without reporting it in the questionnaire. In conclusion, cotinine measurement substantially improved the discrimination between smokers and non-smokers in men with multiple risk factors for cardiovascular disease.

KEY WORDS: Cardiovascular disease. Cotinine. Exposure risk factors. Hypertension. Tobacco.

Introduction

Hypercholesterolaemia, hypertension, diabetes mellitus and smoking are factors associated with increased risk of atherosclerosis.[1] However, while it is easy to measure lipid and glucose levels, it is far more difficult to estimate a patient's tobacco use.

Guidelines for the treatment of hypertension underline the central importance of strenuous efforts to reduce the prevalence of smoking,[1] as epidemiological studies consistently have demonstrated that it increases the risk of cardiovascular disease and death by some two- to three-fold.[1,2] Smoking cessation lessens the risk of death or myocardial infarction in a wide age-group range, as shown in patients with coronary artery disease.[3]

Thus, it is important for the physician to initiate smoking cessation in patients at high coronary risk (i.e. those with hypertension, diabetes mellitus and/or hypercholesterolaemia). However, several population-based studies demonstrate that a majority of smokers, including those with hypertension, believe that physicians are not involved in their efforts to quit smoking.[4,5] One possible explanation for this may be the difficulties associated with establishing the presence – as well as degree and type – of tobacco exposure in the individual patient.[4-6]

Use of smokeless tobacco attracts some attention, and this type of nicotine addiction seems to be associated, at least in part, with health consequences other than those for smoking.[7,8]

With the hypothesis that cotinine measurement substantially will improve discrimination between smokers and non-smokers, the aim of the present study is to examine the relevance of urinary cotinine in establishing tobacco consumption in treated hypertensive men at high coronary risk.

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Materials and methods

Study population

Inclusion criteria were treated hypertension, male sex and at least one of the following factors: tobacco smoking (one cigarette per day or more), diabetes mellitus or serum cholesterol concentration ≥ 6.5 mmol/L. Age ranged from 54 to 77 years, and about 90% of patients were recruited from

two hypertensive cohorts selected randomly from the general population.[9]

At the time of the present investigation, patients had participated for three years in a randomised study that examined the effect of a multifactorial intervention programme directed against smoking, hypercholesterolaemia and diabetes mellitus in treated hypertensive patients.[10] At original inclusion in this longitudinal study, 94 of those presently studied (n=381) were smokers (25%), 294 (77%) had hypercholesterolaemia and 75 (20%) suffered from diabetes mellitus.

Patients were asked to provide overnight (12 h) urine samples and these were used to examine the degree of albuminuria and for determination of urinary cotinine. Samples for cotinine measurement were obtained from 381 out of the 508 patients originally included in the study. Data was unobtainable in 127 cases: 35 patients died and 92 failed to provide a urine sample. However, 81 of the 92 patients completed a questionnaire, in which 21 (26%) reported that they were smokers. This compared with 21% in the total group of 381 patients.

Procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983, and the study was approved by the Ethical Committee of the Medical Faculty at Göteborg University.

Examinations, sampling and measurements

Patient examinations: Patients were examined in the hypertension out-patient department. Current smoking status was assessed by questionnaire[9,10] and from urinary cotinine concentration. Urinary cotinine:creatinine ratio was measured on the sample collected overnight (12 h). Patients who reported as non-smokers but had urinary cotinine concentrations indicating nicotine use according to the decision limits described below were sent a second questionnaire with an accompanying letter. This asked about smoking status, the use of snuff or other smokeless tobacco, if nicotine chewing gum had been used, or the patient lived with a smoker (i.e. passive smoking).

Blood and urine sampling and sample storage: Patients were asked to avoid heavy exercise and caffeine (but were free to smoke) on the evening of the urine collection, and to refrain from eating for 12 h from the evening. Detailed descriptions were given about 12 h urine collection, and the volume was measured when the patient attended the out-patient department the following morning. Samples were frozen (-20°C) immediately for later analysis.

Assays: Urinary cotinine was determined by radioimmunoassay (RIA; nicotine metabolite double antibody, Diagnostic Products Corp, Los Angeles, CA). Cotinine was used for calibration (1.00 mg/L = 5.68 μmol/L) and [^{125}I]cotinine was the radioligand. According to the manufacturer, the highest recorded value for urinary cotinine in 67 non-tobacco users was 1.8 μmol/L, and therefore we used 2.0 μmol/L as the decision limit (the manufacturer used the 2.84 μg/L calibrator to define the cut-off for discriminating tobacco users from non-users). The antiserum cross-reacts with nicotine to about 1%, and with 3-hydroxycotinine to between 15% and 50%, depending on the relative concentration used, whereas high concentrations of a large number of drugs and drug metabolites do not. In 31 consecutive assay runs, the total coefficient of variation was 9.2% at 5.8 μg/L. Creatinine was analysed by a modified Jaffé procedure.

Table 1. Characteristics of the patients

Number	381
Age, years	69.8 ± 4.7
Body mass index, kg/m^2	26.8 ± 3.8
Previous acute myocardial infarction, *n* (%)	38 (10)
Previous stroke, *n* (%)	28 (7)
Intermittent claudication, *n* (%)	38 (10)
Drug treatment, *n* (%)	
Diuretics	117 (31)
β-adrenergic blockers	265 (70)
Calcium antagonists	109 (29)
ACE inhibitor	69 (18)

Means ± SD, if not otherwise stated.

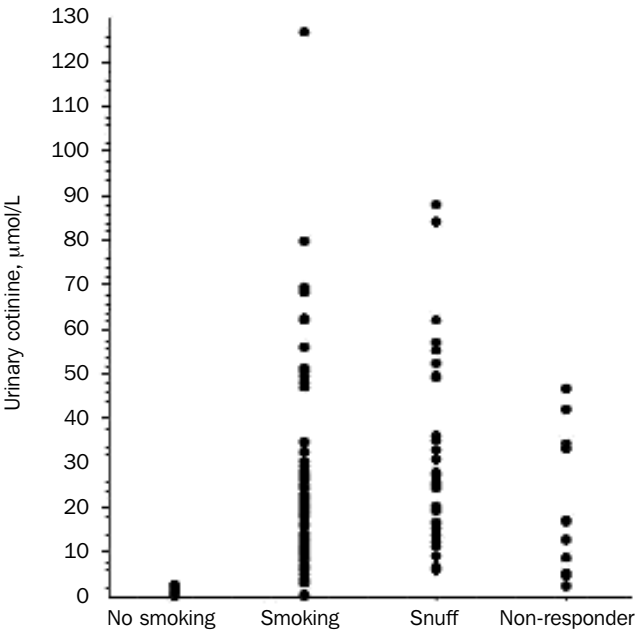

Fig.1. Distribution of urinary cotinine concentrations in relationship to tobacco consumption status as evaluated by questionnaire in treated hypertensive men (non-smoking [n=257], smoking [n=82], snuff using [n=30], non-responders to second questionnaire [n=12]).

Decision limits: Urinary cotinine concentration above 2.0 μmol/L or a urinary cotinine:creatinine concentration ratio over 1.0 mmol/mol was taken to indicate tobacco use.

Definition of tobacco use status: Tobacco use status was defined as one of the following: 1) smoking, use of snuff, nicotine chewing gum or passive smoking, as assessed by questionnaire in combination with elevated urinary cotinine concentration; 2) smoking or other tobacco use, when

Table 2. Comparison between treated hypertensive men who were non-smokers, smokers or snuff-users

	Non-smokers	Smokers	Snuff users
Number	257	94	30
Age, years	69.7 ± 4.7	70.1 ± 4.7	70.2 ± 4.9
Urinary cotinine, μmol/L	0.13 (0.15)	19 (12)*	25 (25)*
Cotinine: creatinine ratio	0.18 (0.03)	2.62 (1.25)*	3.20 (1.83)*§

Results are given as mean ± SD or as median value (50th percentile) in cases of non-parametric distribution.
**Significantly different (P<0.05) with respect to non-smokers.*
§ Significantly different (P<0.05) with respect to smokers.

elevation of urinary cotinine concentration was found in a patient who reported no smoking in the first questionnaire and did not answer the second questionnaire; 3) no smoking, if no reported smoking and no elevation of urinary cotinine concentration; or 4) no smoking, if the patient reported no smoking or no nicotine exposure in the first and second questionnaire and if either urinary cotinine concentration or cotinine:creatinine ratio was not elevated.

Statistical methods

Results are given as mean ± standard deviation or, for variables with markedly skewed distribution, as median values and the interquartile range (50th to 75th percentile). In a first step, continuous variables were compared with ANOVA or Kruskal-Wallis tests. If analysis showed $P<0.05$ then further analyses were performed using Student's *t*-test or Mann-Whitney U-test. Categorical variables were compared using Fisher's exact test.

Sensitivity and specificity of cotinine measurement to discriminate smokers from non-smokers were calculated after exclusion of patients who refused to complete the second questionnaire ($n=12$) and of snuff users ($n=30$). Corresponding calculations on the first questionnaire as an instrument to identify smokers and non-smokers were based on all patients ($n=381$), using urinary cotinine concentration data and the results of the second questionnaire to establish true tobacco use status, according to previously specified definitions. Two-sided $P<0.05$ was regarded as significant.

Results

Patient characteristics are shown in Table 1. Eighty men reported smoking in the first questionnaire. Forty-six men who claimed not to smoke had elevated urinary cotinine concentrations and, in the second questionnaire, were asked about their nicotine consumption. Two patients admitted smoking, 30 reported snuff use and 12 failed to answer (non-responders). Two patients maintained that they did not smoke (urinary cotinine concentrations 2.17 and 2.89 μmol/L, cotinine:creatinine ratios 0.28 and 0.39 mmol/mol, respectively). No patients reported passive smoking or use of nicotine chewing gum (or other nicotine preparations).

Values below the decision limits for urinary cotinine concentration and cotinine:creatinine ratio in smokers ($n=6$) were 0.37 and 0.75 μmol/L, and 0.05 to 0.85 mmol/mol, respectively. Figure 1 compares urinary cotinine concentrations between patient groups. Values for patients who did not complete the second questionnaire are given in the non-responder group.

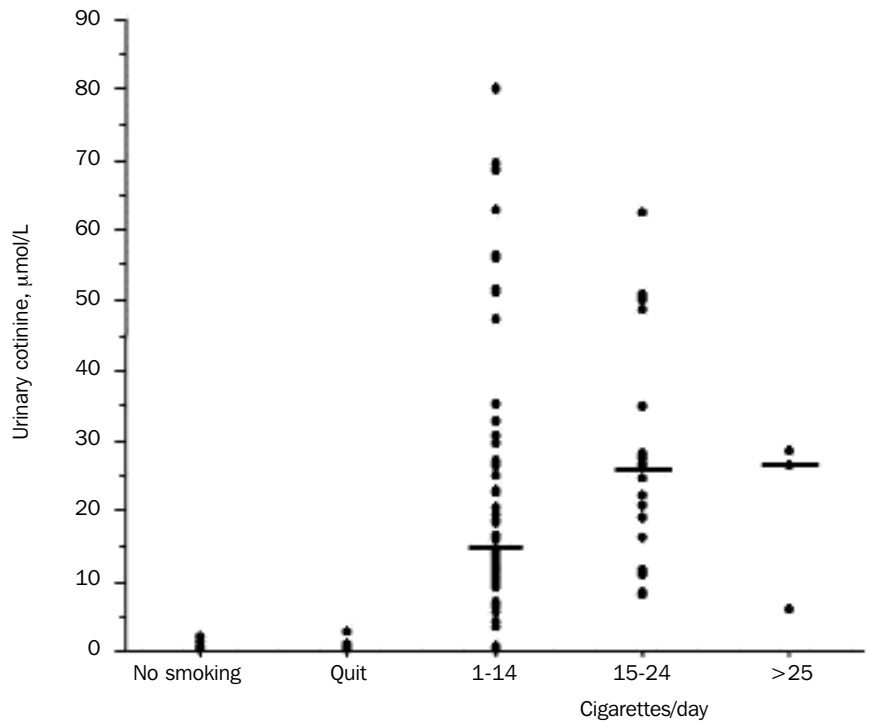

Fig. 2. Urinary cotinine concentrations in relation to smoking status as evaluated by questionnaire in treated hypertensive men (non-smokers [$n=126$], smokers who had quit [$n=131$], smokers of 1-14 cigarettes/day [$n=57$], 15-24 cigarettes/day [$n=20$] and >24 cigarettes/day [$n=3$]). Patients with elevated urinary cotinine concentrations who used snuff ($n=30$), who did not answer a second questionnaire about their exposure to nicotine ($n=12$) or who did not specify how much they smoked ($n=2$) were excluded. Horizontal bars indicate median values ($P<0.0001$, Kruskal-Wallis test).

Median values (interquartile range) for urinary cotinine concentration were 17.2 (18.3) μmol/L and 19.3 (10.5) μmol/L in the non-responder and smoking groups, respectively (P=0.75). In 10 out of the 12 patients in the non-responder group, cotinine:creatinine ratios were elevated (not shown in Figure 1). For non-responders, self-reported smoking status three years prior to the present study can be broken down as follows: five were non-smokers, five had quit smoking and two were smokers.

Relationship between daily tobacco usage and urinary cotinine excretion was analysed after exclusion of patients in whom data on intake levels were missing (i.e. snuff users [n=30], non-responders [n=12] and those who admitted smoking but did not specify how much [n=2]). Results are shown in Figure 2 and indicate a graded relationship (P<0.0001, Kruskal-Wallis test). However, the ranges between lowest and highest urinary cotinine values were high in the subgroups: 80 μmol/L and 55 μmol/L, respectively, in the groups that reported daily tobacco consumption of 1-14 cigarettes and 15-24 cigarettes.

Measurement of urinary cotinine concentration showed a sensitivity and specificity in distinguishing between smokers and non-smokers of 98 % and 99 %, respectively. Corresponding figures for urinary cotinine:creatinine ratio were 93% and 100%, respectively.

The first questionnaire showed a sensitivity of 85% and a specificity of 100%. In 14 (15%) out of 94 patients, cotinine data indicated the possibility of tobacco consumption, although this was not reported in the questionnaire. Subsequently, two patients admitted to being smokers.

Smokers, non-smokers and snuff users are compared in Table 2. Differences in urinary cotinine concentration and cotinine:creatinine ratio between non-smokers and the groups that used tobacco were highly significant.

Discussion

Results show that urinary cotinine determination substantially improved the possibility of distinguishing between those who used tobacco and those who did not in this group of hypertensive men at high coronary risk. With the decision limits used, sensitivity and specificity rates exceeded 97% – results that support the findings of other studies.[6,11] Adjusting urinary cotinine concentration for creatinine concentration did not increase diagnostic sensitivity.

Cotinine measurement proved not to be 100% sensitive in identifying active smokers, and not completely successful in identifying non-smokers. A problem with the interpretation of the figures for the diagnostic performance of the urinary cotinine determination, however, is the reliability of the questionnaire. For example, the study showed 15% under-reporting of tobacco exposure, which was only revealed after continued questioning of patients with high urinary cotinine excretion.

Complete concordance between positive cotinine test and patients' smoking habits cannot be expected. First, passive smoking may cause a measurable uptake of nicotine. Such indirect smoking appears to be associated with a 30% increase in cardiovascular risk.[12] Hence, determination of cotinine may be of value in such cases, although patients may be misclassified as active tobacco consumers. Second, the patient may be a user of smokeless tobacco, which is common in several countries.[7,8,13] Finally, there is an increasing number of different nicotine preparations, developed to reduce the symptoms associated with withdrawal from smoking. Such agents may also confound cotinine measurement, when applied to detect tobacco use. Naturally, the degree of smoking varies, which may explain the normal cotinine values in two reported smokers.

None of the patients who initially reported non-smoking status, but had urinary cotinine concentrations above the decision limit, reported exposure to a smoking environment or use of nicotine chewing gum. Instead, they admitted smoking, reported use of snuff, or gave no answer. The group that gave no answer in the final questionnaire was characterised as smokers – all had high cotinine concentrations, and cotinine:creatinine ratio was elevated in 10 cases out of 12. In addition, the median urinary cotinine concentration in this group was similar to that in the smoking group. Experiences from previous studies, and the similarity in values in known smokers, makes it highly unlikely that these were false-positive results.[6,11] This notwithstanding, the test is a valuable tool to distinguish between those who use tobacco and those who do not, and seems to be the best method available at present to identify tobacco use.[6,11]

Cotinine is a metabolite of nicotine and has a half-life of elimination from the blood of approximately 20 hours.[13] In the present study, cotinine was measured in an overnight urine collection sample, which may have proved more accurate than analysis of a random sample of urine or saliva. Some 8% of the patients were snuff users, but did not smoke, and the finding that this group had higher cotinine levels than smokers is in agreement with previous observations.[8,14] Thus, Swedish users of smokeless tobacco in general are exposed to a nicotine dose approximately 65% higher than is the average smoker.[8] Despite this difference, a recent study has shown that snuff is associated with a lower risk for myocardial infarction than smoking,[7] although a large cross-sectional study indicated an association between cardiovascular disease and the use of snuff.[8] Perhaps there are constituents in tobacco smoke that are of greater importance in the disease process than nicotine alone.

Determination of urinary cotinine concentration proved a valuable tool in distinguishing tobacco users and non-users, improving the sensitivity of a questionnaire from 85% to 98%. Previous smoking cessation programmes, within the framework of multifactorial risk-factor intervention studies, in general have only used questionnaires to establish smoking habits,[15,16] or tests with lower sensitivity and specificity, such as thiocyanate determination.[17]

In future studies concerning tobacco consumption in this category of patient, cotinine measurement should be used as an objective marker. □

This study was supported by grants from the Swedish Medical Research Council (B92-19X-09937-01A, B93-19X-09937-02B, B93-19X-03506, B94-19X 09937-02B), the Swedish Heart and Lung Foundation, the Swedish Hypertension Society, and King Gustaf V and Queen Viktoria foundation. Thanks are due to Diagnostic Products Corp., Los Angeles, CA, for supplying reagents for urinary cotinine determination.

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