

Assessment of exposure to tobacco smoke: measurement of exhaled carbon monoxide and hair nicotine

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Aim: To investigate the effect of tobacco smoke (TS) exposure on the quantity of exhaled carbon monoxide (eCO) and hair nicotine (HN) and to evaluate the relationship between these values.

Materials and methods: Included in the study were 96 subjects (64 male, 32 female) divided into 3 groups. The subjects in Group 1 (n = 46) were current smokers, and the subjects in Group 2 (n = 20) and Group 3 (n = 30) were nonsmokers with or without environmental TS exposure, respectively. The eCO level of all of the subjects was measured with a breath CO monitor. Gas chromatography/mass spectrometry were used for quantification of the HN (n = 47).

Results: The mean age of the subjects was 39.1 years. The mean levels of eCO were 9.3 ppm, 1.3 ppm, and 1.0 ppm and the mean HN concentrations were 20.9 ng/mg, 2.1 ng/mg, and 0.7 ng/mg in the 3 groups, respectively. There was a significant difference between Group 1 and the other groups according to the levels of eCO and HN concentrations, but the levels of eCO and HN concentrations were similar in Group 2 and Group 3. There was a positive correlation between the levels of eCO and the HN concentrations. The cutoff values of eCO and HN for smokers were 6 ppm and 4 ng/mg, respectively.

Conclusion: Although nicotine analysis in some biological samples like hair is specific to TS exposure, these methods are expensive and difficult procedures. Our results suggest that instead of HN analysis, a cheap and easy method like eCO measurement may be used, but further studies with more cases are needed.

Key words: Environmental tobacco smoke, exhaled carbon monoxide, nicotine, hair nicotine, secondhand smoke, tobacco smoke

Tütün dumanı maruziyetinin değerlendirilmesi: Soluk havasında karbonmonoksit ve saçta nikotin ölçümü

Amaç: Tütün dumanı maruziyetinin soluk havasındaki karbonmonoksit (eCO) ve saçtaki nikotin düzeyine etkisini saptamayı ve bu yöntemler arasındaki ilişkiyi belirlemek.

Yöntem ve gereç: Çalışmaya 3 grup olarak 96 olgu (64/32 erkek/kadın) çalışmaya alındı. Grup 1 (n = 46) sigara içicisi idi, Grup 2 (n = 20) ve Grup 3 (n = 30) sigara içmeyen ancak çevresel tütün dumanı olan ya da olmayan olgulardı. Tüm olgularda CO monitörü ile eCO düzeyi ölçüldü. Saçta nikotin konsantrasyonunu belirlemede (n = 47) gaz kromatografi/kütle spektrometri yöntemi kullanıldı.

Bulgular: Olguların yaş ortalaması 39,1 idi. Ortalama eCO düzeyi gruplarda 9,3 ppm, 1,3 ppm ve 1,0 ppm ve saçta nikotin konsantrasyonu sırasıyla 20,9 ng/mg, 2,1 ng/mg ve 0,7 ng/mg idi. eCO düzeyleri ve saçta nikotin konsantrasyonu açısından Grup 1 ile diğer gruplar arasında farklılık saptandı ancak eCO düzeyleri ve saçta nikotin konsantrasyonu.

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Grup 2 ve Grup 3'de benzerdi. eCO düzeyleri ile saç nikotin konsantrasyonları arasında pozitif ilişki saptandı. Sigara içicilerinde eCO ve saçta nikotin için eşik değerleri sırasıyla 6 ppm ve 4 ng/mg idi.

Sonuç: Saç gibi çeşitli biyolojik örneklerde nikotin analizi tütün dumanı maruziyeti için spesifik olmasına rağmen bu yöntemler pahalı ve zordur. Sonuçlarımız saç nikotin analizi yerine eCO ölçümü gibi ucuz ve kolay bir yöntemin kullanabileceğini desteklese de daha fazla sayıda olgu içeren yeni çalışmalara gereksinim vardır.

Anahtar sözcükler: Çevresel tütün dumanı, soluk havası karbonmonoksiti, nikotin, saçta nikotin, pasif içicilik, tütün dumanı

Introduction

Smoking is the most widespread addiction, affecting about one-third of the world's population, and it is well recognized that cigarette smoking is the primary preventable cause of death (1-3).

Tobacco smoke (TS) is a complex mixture of chemicals. Three chemical constituents of TS are carbon monoxide (CO), hydrogen cyanide, and nicotine. These chemicals are absorbed and can be detected as intact compounds or as metabolic products (4).

CO is one of the most toxic agents in TS and it is present in mainstream and sidestream smoke. Exposure to CO can be assessed as CO in expired alveolar air. The exhaled CO (eCO) is one of the most commonly used markers to quantify TS exposure. The most likely cause of high levels of CO exposure is smoking, and increased levels of eCO reflect the degree of TS exposure of the lungs. Other factors leading to CO exposure are environmental pollution, passive smoking, and occupational exposure (4,5).

Cotinine is the primary metabolite of nicotine and it can be measured in urine, blood, and saliva. Urinary measurement is the most useful for the follow-up of smoking cessation (4,6). The first paper concerning hair nicotine (HN) determination was published in 1983 by Ishiyama et al (7). Since then, the examining of HN content has become a valuable tool facilitating the assessment of exposure to TS. HN reflects a gradual accumulation over a long period. Each centimeter of hair represents about 1 month of cumulative TS exposure (8,9). Although they are costly and technically difficult methods, quantitative nicotine analyses have the advantage of being specific to TS exposure (8). In this study, both current and passive smokers were evaluated. Passive smoking is also called secondhand smoking

(SHS), environmental tobacco smoking (ETS), or involuntary smoking. SHS involves the smoke of a smoldering tobacco product and the exhaled breath of a smoking individual, and SHS exposure is a worldwide public health problem (10,11).

The measurement of SHS exposure may be useful in identifying occupational risks resulting in potential health problems. eCO, cotinine, and nicotine are useful biomarkers for measuring SHS exposure (12-16).

The passive smoking rate in Turkey was reported as 67.0%-97.4% (17,18). The SHS rate can be determined more accurately by measuring the concentration of nicotine in hair, cotinine in urine, or eCO. In this study, we aimed to detect and compare the HN concentrations and eCO levels in active and passive smokers and subjects without TS exposure, and to determine the cutoff values for these markers in smokers and nonsmokers.

Materials and methods

Subjects

A total of 96 healthy subjects (64 male, 32 female) were enrolled in the study. The subjects were divided into 3 groups according smoking status and SHS, and none of the subjects worked in a pub, bar, cafe, or Turkish coffeehouse (19), which could cause intensive exposure to TS.

Group 1 (n = 46) consisted of current smokers.

Group 2 (n = 20) consisted of people who were nonsmokers within the last year but who had SHS exposure of more than 3 h daily for at least 1 year in some microenvironments, including their homes, neighbor's homes, workplaces, or Turkish coffeehouses.

Group 3 (n = 30) consisted of people who were nonsmokers within the last year, without any SHS exposure.

The Fagerström Test for Nicotine Dependence (FTND) was administered to all of the smokers.

The study protocol was approved by the local ethics committee of Gaziosmanpaşa University Hospital and informed consent was obtained from all of the participants.

Exhaled CO measurement

eCO levels were measured by the single breath method using a breath carbon monoxide monitor (Micro 4 Smokerlyzer, Bedfont Scientific Ltd., UK). The subjects were instructed to take a deep breath, hold their breath, and exhale fully into the mouthpiece of the detector.

Hair sampling

Undyed or unbleached hair samples that had retained their natural color were used. The hair from the vertex of the scalp was cut as close to the skin as possible and placed in a paper envelope. The collected samples were stored at 4 °C.

Nicotine extraction in hair

The analytical method was an adaptation of the procedure used by Zahlsen and Nilsen in 1990 (20). The hair samples (8-10 mg) were incubated in 5 M NaOH (3 mL) (Climax, Turkey) at 30 °C for 24 h. After incubation, 1 mL of 99% dichloromethane solution (Merck, Germany) was added to each sample and samples were then vortexed for 2 min. The upper organic phase was separated and then 100 µL of 99.5% n-butanol solution (Merck) was added to make a final volume of 100 µL. The solvent was removed by nitrogen gases, transferred to a vial using an inserter, and analyzed by gas chromatography-mass spectrometry (GC-MS). The simplified GC-MS assay is sensitive and applicable for routine screening of chronic SHS exposure in population-based epidemiologic studies (21). Analyses were carried out using a PerkinElmer Clarus 500 gas chromatograph/mass spectrometer (USA). The GC-MS oven temperature program was regulated as follows: the initial temperature was set at 50 °C and raised at a rate of 5 °C/min up to 180 °C, which was maintained for 4 min. Afterwards, the temperature

was raised again at a rate of 20 °C/min to 250 °C and held at that level for 10 min. As a carrier gas, 5 µL of helium at a rate of 1 mL/min was injected into the columns using a splitless mode. The column BPX5 had a film thickness of 30 m × 0.25 mm × 250 µm. For the quantification of nicotine in hair samples, concentrations of standard nicotine at 0.01 ppm, 1.00 ppm, and 10.00 ppm were analyzed by GC-MS. A calibration curve with an R² value of 0.99948 was obtained. Quantitative determination of nicotine was carried out based on peak area measurements.

Statistical analysis

All of the continuous variables had normal distribution according to the Kolmogorov-Smirnov normality test. One-way ANOVA and 2 independent sample t-tests were used to compare the continuous variables. For multiple comparisons, the least significant differences test was used. Continuous variables were presented as arithmetic means (mean) and standard deviations (SD). Correlation analysis was used to determine the relation of eCO and HN with smoking properties. Receiver operating characteristics (ROC) curve analysis was used to determine the cutoff value of CO according to smoking status. P < 0.05 was considered statistically significant. Statistical analysis was performed using commercially available software (PASW 18, SPSS Inc., USA).

Results

A total of 96 subjects (64 male, 32 female) in 3 groups were included in the study. The mean age of all of the subjects was 39.1 ± 12.0 years.

Group 1 included 46 subjects (35 male, 11 female) with a mean age of 36.1 ± 8.3 years, who had smoked 20.6 ± 11.8 pack-years. Twenty (43.5%) had smoked ≥20 pack-years, and 26 (56.5%) smoked more than 20 cigarettes per day. The mean FTND score was 6.4 ± 2.0.

Group 2 included 20 subjects (14 male, 6 female) with a mean age of 37.4 ± 10.1 years.

The mean age of the subjects in Group 3 (14 male, 12 female) was 44.8 ± 15.7 years.

The eCO levels of all of the subjects were measured and the levels were higher in the smokers than in the

nonsmokers. The eCO levels were similar in Group 2 and Group 3 (Table). The HN concentrations of 47 of the subjects (18 subjects in Group 1, 15 subjects in Group 2, and 14 subjects in Group 3) were measured. The mean HN concentration was higher in Group 1 than in the other groups, but similar in Group 2 and Group 3 (Table).

The mean levels of eCO and HN were similar in the smokers who smoked ≥ 20 cigarettes per day compared to those who smoked < 20 cigarettes per day. No significant correlation between the levels of eCO and HN and the number of cigarettes smoked per day, pack-years, or FTND scores were found among the smokers.

A significant positive correlation was found between the levels of eCO and the concentration of HN ($r = 0.432, P < 0.002$) (Figure).

According to the ROC curve analysis, the cutoff value for eCO was determined as 1.5 ppm for nonsmokers and 6 ppm for smokers. The cutoff value of HN concentration, which distinguishes smokers from nonsmokers, was 4 ng/mg.

Discussion

In this study, the HN concentration and eCO level in different groups was investigated with the aim of determining TS exposure. The eCO levels were higher in the smokers than in the other groups, regardless of the amount of cigarettes smoked per day. The concentrations of HN were also higher in

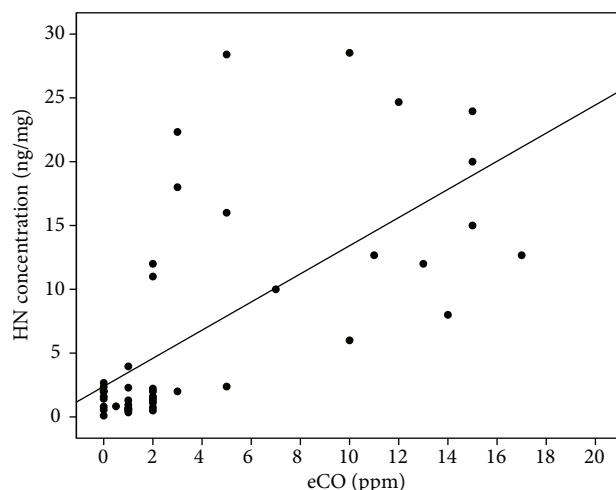


Figure. Correlation between the levels of eCO and HN concentration.

the smokers than in the other groups. However, the eCO and HN levels were not sufficiently different to distinguish subjects with or without SHS exposure.

It was reported that smokers had higher mean HN levels than nonsmokers (12,21-23). In the literature, there are different results for the HN levels of smokers (33.9 $\mu\text{g/g}$, 38.3 $\mu\text{g/g}$, 30.6 $\mu\text{g/g}$, and 39.0 ng/mg) and nonsmokers (0.006 $\mu\text{g/g}$, 1.8 $\mu\text{g/g}$, 4.5 $\mu\text{g/g}$, and 2.5 ng/mg) (19,22,24,25). Man et al. reported mean concentrations of HN in active smokers, SHS-exposed nonsmokers, and unexposed nonsmokers as 26.3 ng/mg, 2.9 ng/mg, and 1.0 ng/mg (21). In comparison, in the present study, a slightly lower HN concentration was determined. In smokers,

Table. HN and eCO levels in the study groups.

	HN (ng/mg) (mean \pm SD)	P	eCO (ppm) (mean \pm SD)	P
Group 1	20.89 \pm 19.65		9.3 \pm 5.1	
Group 2	2.07 \pm 0.67	<0.001	1.3 \pm 1.3	<0.001*
Group 3	0.7 \pm 0.3		1.0 \pm 0.8	
Smoker	20.89 \pm 19.65		9.3 \pm 5.1	
Nonsmoker	1.41 \pm 0.87	<0.001	1.1 \pm 1.0	<0.001

* According to pairwise comparison, there was a statistically significant difference between smokers and the other 2 groups. The HN concentrations were similar in Group 2 and Group 3.

the minimal and maximal HN concentrations were 6.0 ng/mg and 94.8 ng/mg, respectively. The large variations in nicotine concentrations may be due to many different factors such as exposure to SHS, exposure time, distance from the source of exposure, and differences in ventilation status (26,27).

Kintz et al. reported that 2 ng/mg of HN can be used to distinguish smokers from nonsmokers (22). In another study, the cutoff value was defined as 2-5 ng/mg HN for smokers (23). In our study, the cutoff value of HN concentration for smokers was within this range. The concentrations of HN were similar in subjects exposed or unexposed to SHS. It should be noted again that none of the subjects work in a pub, bar, cafe, or Turkish coffeehouse, which would cause intensive exposure to TS. Moreover, this result may be associated with some individual properties like hair growth, color, and nicotine absorption, which affect the concentration of HN (8,26-28); the duration and intensity of exposure of TS; the distance from the source of the TS; and differences in ventilation status.

Prior studies have found that HN level is associated with the number of cigarettes smoked per day. Eliopoulos et al. reported that higher HN levels were associated with an increased number of cigarettes smoked per day (29). Our results did not support their findings; namely, we could not detect an impact of the number of cigarettes smoked per day on HN concentrations, as was also the case in a study by Al-Delaimy et al. (30). This result may be associated with individual properties of hair and the intensity and duration of exposure, as noted previously. The range variation of HN concentrations of smokers may be another reason for the similar finding.

eCO is one of the most common measures used to quantify tobacco exposure (4). It is used to confirm smoking status in smoking cessation programs (31). The mean levels of eCO were reported as 9.5-21.6 ppm in smokers and 1.3-4.3 ppm in nonsmokers (16, 13,31-33). In our study, the mean levels of eCO were 9.3 ppm in smokers and 1.1 ppm in nonsmokers. Although it is used to confirm smoking status, the cutoff level is still a matter of debate (31). Middleton et al. (13) and Deveci et al. (16) proposed the use of 6 ppm and 6.5 ppm as the cutoff breath CO levels in smokers and nonsmokers, respectively. We determined similar cutoff values.

In different studies, the levels of eCO in passive smokers were reported as 1.2-3 ppm (33-36). These different results may be associated with the difference in exposure time, the number of smokers implicated for SHS exposure, the distance from the source of the exposure, and ventilation status. In our study, the mean level of eCO in cases with ETS exposure was compatible with those found in the literature.

The eCO level depends on the number of cigarettes smoked per day (37,38). Deveci et al. reported a significant positive correlation between eCO levels and daily cigarette consumption (16). In our study, there was no correlation between the levels of eCO and the number of cigarettes smoked per day. Although Temel et al. found a positive correlation in smokers of pack-years and FTND scores with the levels of eCO, we did not find same relations (35).

We found a positive correlation between eCO levels and HN concentrations, and we could not find any study to evaluate these 2 parameters in the current literature. However, Underner et al. reported a significant correlation between eCO and urinary cotinine (39). Fritz et al. determined that eCO measurements are nearly as sensitive as the urinary cotinine level for detecting smoking status (40).

Although nicotine analysis in some biological samples like hair is specific to TS exposure, these methods are expensive and difficult procedures. In our study, the correlation between the levels of eCO and HN concentrations suggests that instead of HN analysis, a cheap and easy method like eCO measurement may be used, but further studies with more subjects are needed.

For distinguishing smokers and nonsmokers, a HN concentration of 4 ng/mg and an eCO level of 6 ppm can be used as cutoff values.

We could not find any effect of TS exposure in nonsmokers on the quantities of eCO and HN, but it should be noted again that none of these subjects worked in a pub, bar, cafe, or Turkish coffee house, which can cause intensive exposure to TS.

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