Effects of smoking cessation on biological monitoring markers in urine

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Abstract

Introduction: Urinary nicotine and cotinine levels are often measured as biomarkers for tobacco smoke exposure. However, these biomarkers are not appropriate to evaluate the effects of quitting smoking for several days, because of their short half-lives. In this study, we focused on the changes in the urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) levels of 55 patients in a smoking cessation program, because of the long half-life. At the same time, urinary 7-methylguanine (m7Gua) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), as DNA damage markers of cigarette smoking, were also measured.

Results: In the subjects who completed the quit-smoking program (18 subjects out of 55), the urinary nicotine and cotinine levels decreased to 1.7 and 0.2% at 8 weeks after the first visit to the clinic. By contrast, the NNAL levels decreased to 12.3% at 8 weeks after quitting smoking. During the same period, the urinary m7Gua levels significantly decreased, from 27.32 μg/mg creatinine to 14.17 μg/mg creatinine by the elimination of subjects who showed increased levels of NNAL during the smoking cessation program. The 8-OHdG levels were also reduced within the same period, but were not significantly different. From the all data analysis, the urinary levels of cotinine and NNAL positively correlated with the level of m7Gua.

Conclusions: NNAL may be an appropriate exposure marker for evaluating the smoking status of patients in a smoking cessation program. The urinary cotinine and NNAL levels positively correlated with the m7Gua levels.

Keywords: Nicotine, Cotinine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), 7-methylguanine (m7Gua), 8-hydroxy-2′-deoxyguanosine (8-OHdG), Smoking cessation

Introduction

Tobacco smoke contains more than 5000 chemicals and over 70 types of carcinogens [1, 2]. Smoking has been established as a risk factor for many common cancers [3]. The percentage of smokers is decreasing each year in Japan (from 24.2% in 2006 to 17.8% in 2018); however, it still remains high, especially among men (29.0% in men vs. 8.1% in women) [4]. Smoking cessation is one of the most effective interventions to prevent cancer. At smoking cessation programs in hospitals and clinics, the abstinence status is usually biochemically confirmed by an expiratory carbon monoxide (CO) concentration below 9 ppm. However, due to the short half-life of CO (2 h), many people who smoked over 24 h before the test could be misclassified as quitters [5]. In addition to CO, biochemical verification of tobacco abstinence can be obtained by measuring the cotinine levels in urine, plasma and saliva. Cotinine is the main metabolite of nicotine and has a longer half-life (18 h) than CO and nicotine (2 h) [6]. In measurements of tobacco use, cotinine has relatively high sensitivity and specificity, as compared to CO levels. Therefore, it is a reliable indicator of recent nicotine intake [7]. However, for patients

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undergoing nicotine replacement therapy (NRT) using nicotine gum and nicotine patches, cotinine cannot be used as an indicator of smoking cessation because nicotine and cotinine are present at the same levels as smokers [8–12]. On the other hand, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine has been reported as an exposure indicator that is not affected by NRT [13, 14]. NNAL is a tobacco-specific nitrosamine and a metabolite of the carcinogenic 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in humans [3]. Many studies have investigated the urinary NNAL levels in smokers and secondhand smokers [15, 16]. As urine contains quantitatively significant NNK metabolites, the NNAL levels in urine are critically useful in studies of human exposure to tobacco smoke. Urinary NNAL has a much longer half-life (10–40 days) than urinary cotinine and can be detected even a few weeks after smoking [6]. Therefore, NNAL is a possible candidate for an indicator of quitting smoking. However, only a few studies have used urinary NNAL as an indicator of smoking cessation in clinical settings.

Tobacco smoke contains many carcinogens that can lead to DNA methylation and oxidation. A metabolite of NNK and NNAL, methanediazonium ion, reacts with DNA to form methyl DNA base adducts, including 7-methylguanine (m³Gua) and O⁶-methyl deoxyguanine [17]. m³Gua is removed from DNA by a glycosylase to produce an apurinic site [18]. As the result, m³Gua is excreted into the urine. The apurinic site is a frequent cause of mutations in mammalian cells [19, 20]. Current smokers had higher m³Gua levels in their lung DNA [21]. A correlation between urinary m³Gua levels and urinary NNAL levels has been reported [22].

Oxidative damage of DNA by reactive oxygen species leads to the production of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a specific biomarker of oxidative stress [23–25]. Several studies reported that urinary 8-OHdG levels [26–28] and salivary 8-hydroxyguanine levels [29, 30] correlated with smoking. Cao et al. [31] reported that the levels of 8-OHdG in bronchoalveolar lavage fluid were associated with the Tumor Node Metastasis stage, indicating that oxidative DNA damage is a marker for the development of lung cancer.

The purpose of this study is to investigate the reductions of cigarette smoking exposure markers (nicotine, cotinine and NNAL) and DNA damage markers (m³Gua and 8-OHdG) in patients participating in a smoking cessation program.

Materials and methods

Subjects and urine sample collection

A total of 55 subjects (36 male and 19 female, ages 25–68) from a smoking cessation clinic in Japan participated in the study. After excluding samples because of only a single visit or the use of only heated tobacco products, a total of 42 subjects (26 male and 16 female) were selected for the analysis (Table 1).

Subjects visited the clinic 2 to 5 times during the 12-week treatment period, and urine samples were collected at the first visit and at weeks 2 and 8 after quitting smoking. The number of subjects who participated up to the second visit (2 weeks) was 42 (Group 1; 26 male and 16 female). The number of subjects who participated up to the third visit (8 weeks) was 18 (Group 2; 11 male and 7 female). At each clinic visit, 10 mL urine samples were collected in polypropylene centrifuge tubes and stored at −20 °C until analysis. The study protocol was approved by the Ethics Committee of Medicine and Medical Care, University of Occupational and Environmental Health, Japan.

Chemicals

(−)-Nicotine (≥98%), (−)-cotinine (≥98%), and Type H-1 β-glucuronidase (208,400 units/g solid) were obtained from Sigma Aldrich Inc. (St. Louis, MO). Isolute SLE+ column cartridges were purchased from Biotage (Uppsala, Sweden). Chloroform (≥99.7%) and acetic acid (≥99.7%) were purchased from FUJIFILM Wako Pure Chemicals Co., Inc. (Osaka, Japan). Acetonitrile (≥99.8%), 1 mol/L ammonium acetate solution (LC/MS grade) and distilled water (LC/MS grade) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). NNAL (100 μg/mL in acetonitrile), DL-methyl-D₃-cotinine (100 μg/mL in acetonitrile), and 1, 2, 3′, 4′, 5′, 6′-13C₆-NNAL (100 μg/mL in acetonitrile) were obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA). The stock solutions (each 100 μg/mL) of nicotine, cotinine, and NNAL were prepared in acetonitrile. DL-methyl-D₃-cotinine (10 μg/mL) and 13C₆-NNAL (20 ng/mL) stock solutions were also prepared in acetonitrile. The working solutions were prepared using serial dilutions of the stock solutions with a 10% (v/v) acetonitrile solution containing 10 mM ammonium acetate.

Sample preparation

Frozen urine samples were thawed at room temperature. Urine (500 μL) was mixed with 500 μL of acetate buffer (50 mM, pH 4.0), followed by the addition of 2 μL of DL-methyl-D₃-cotinine and 13C₆-NNAL stock solutions as internal standards. β-Glucuronidase (1000 U/500 μL of urine) was then added and the solution was incubated at 37 °C for 15 h. The mixture was loaded onto Isolute

| Table 1 Participants in this study | Mean ± SD |
|-----------------------------------|-----------|
|                                   | Male      | Female   | Total   |
| Ages (years)                      | 44.6 ± 12.7 | 42.8 ± 10.3 | 43.9 ± 11.8 |
| n                                 | 26        | 16       | 42      |
SLE+ column cartridges (Biotage) and allowed to adsorb on the diatomaceous earth supported material for 10 min, followed by elution with 6 mL of chloroform. The extract was evaporated to dryness at 40 °C under a continuous flow of nitrogen. The residue was dissolved in 200 μL of a 10% (v/v) acetonitrile solution containing 10 mM ammonium acetate. The solution was filtered through a pretreatment filter. The filtered solution was divided into 100 μL aliquots. One was diluted 5–20-fold with a 10% acetonitrile solution containing 10 mM ammonium acetate, for the measurement of urinary nicotine and cotinine. The other was used for urinary NNAL measurement without dilution.

Liquid chromatography and mass spectrometry conditions
Analyses of urinary nicotine, cotinine and NNAL were conducted using an HPLC (UltiMate 3000, Thermo Fisher Scientific, Yokohama, Japan) coupled to a hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific Q Exactive Focus) with heated electrospray ionization (HESI-II). The sample separation was achieved on an Acclaim® 120 C18 (2.1 mm × 50 mm, 3 μm, Thermo Scientific, Sunnyvale, CA) column with a flow rate of 0.3 mL/min and a column temperature of 30 °C. Mobile phase A was 10 mM ammonium formate and mobile phase B was acetonitrile. The ion spray voltage was set to a heater temperature of 300 °C. Mobile phase A was set to a gas and auxiliary gas pressures were set to 50 and 15 arbitrary units, respectively. The ion spray voltage was set to 2.5 kV, with a capillary temperature of 250 °C, and the S-lens RF level was 40. Data were acquired in the PRM mode, a single precursor ion [M + H] + was selected in parallel reaction monitoring (PRM) mode. In this mode, the most abundant ion was detected by a Gilson UV detector (UV/VIS-155 with 0.2 mm light path cell). Creatinine and m’Gua were detected at 235 and 305 nm, respectively. The 8-OHdG fraction was collected, depending on the relative elution position from the peak of the added marker, 8-OHG, and was automatically injected into the HPLC-2 column. The 8-OHdG fraction was fractionated by the HPLC-2 column (GL Science Inc., Inertsil ODS-3, 3 μm, 4.6 × 250 mm; elution, 10 mM sodium phosphate buffer [pH 6.7] containing 5% methanol and an antiseptic reagent MB [100 μL/L], 1 mL/ min, 45 °C). The 8-OHdG was detected by a Coulombec II EC detector (ESA Inc., Chemsford, MA, USA) with a guard cell (5020) and an analytical cell (5011) (applied voltages: guard cell, 350 mV; E1, 100 mV; E2, 300 mV).

Statistical methods
The values of each biomarker were compared with the median, because the data did not follow a normal distribution. Statistical analyses were performed using GraphPad Prism, version 7.04 (GraphPad Software, San Diego, CA, USA). Data were tested for normality using Shapiro-Wilk’s test. Non-parametric tests were used because all variables were not normally distributed. Two-sided p values less than 0.05 were considered significant.

Results
Detection sensitivity of urinary tobacco exposure markers
The lower detection limits for nicotine, cotinine, and NNAL were determined to be 0.79 ng/mL, 0.21 ng/mL, and 1.85 pg/mL, respectively. The lower limits of quantification for nicotine, cotinine, and NNAL were 2.62 ng/mL, 0.71 ng/mL, and 6.17 pg/mL, respectively. The correlation coefficients (r²) were > 0.99 in all cases. Representative chromatograms of these exposure markers in urine from a smoker are shown in Fig. 1.
Urinary biomarker levels after smoking cessation

Urinary levels of cigarette smoking markers decreased clearly with time after smoking cessation for 2 and 8 weeks (Fig. 2). Among the subjects who completed the quit-smoking program (Group 2 in Fig. 2), the median values of the urinary nicotine levels (minimum-maximum) at the initial visit to the tobacco cessation clinic, and at 2 and 8 weeks later were 477.2 (36.3–2226.5) ng/mg creatinine, 8.7 (not detected (n.d.) – 264.9) ng/mg creatinine, and 8.2 (1.9–263.1) ng/mg creatinine. The urinary cotinine levels at the same times were 3006.0 (496.4–14,084.5) ng/mg creatinine, 102.7 (2.3–969.8) ng/mg creatinine, and 6.1 (n.d. - 2803.7) ng/mg creatinine. The urinary NNAL levels were 171.7 (31.0–700.9) pg/mg creatinine, 53.9 (10.9–151.4) pg/mg creatinine, and 21.1 (4.4–174.0) pg/mg creatinine. The nicotine, cotinine, and NNAL levels are represented as the total of the free and glucuronidated forms. The individual changes of each biomarker are shown in Fig. 2d-f. Looking at each individual, the urinary NNAL levels of 9 subjects (5 subjects in Group 1 and 6 subjects in Group 2, two subjects overlapped) increased at some points during smoking cessation, as compared to the previous medical examination. Although the rates of the NNAL increases were not markedly high, in order to evaluate the effects of quitting smoking on the DNA damage markers, those subjects were eliminated from the following group analysis and monitored separately. Consequently, the DNA damage markers in urine, as the early adverse health effect markers, were analyzed separately for those 9 individuals. In the analysis excluding those subjects, the DNA damage markers in urine decreased with time after smoking cessation (Fig. 3). In the subjects who completed the smoking cessation program (Group 4 in Fig. 3), the median values (with minimum-maximum) of the urinary m7Gua levels at the initial visit to the tobacco cessation clinic, and at 2 and 8 weeks later were 27.32 (8.21–41.30) μg/mg creatinine, 16.17 (6.05–58.94) μg/mg creatinine, and 14.17 (6.02–47.06) μg/mg creatinine. The urinary 8-OHdG levels at the same times were 5.21 (2.61–8.60) ng/mg creatinine, 4.75 (2.85–6.87) ng/mg creatinine, and 5.09 (2.14–9.20) ng/mg creatinine, respectively. In the case of all subjects (Group 3 in Fig. 3), even discontinued subjects were included, and their urinary m7Gua and 8-OHdG levels were significantly decreased at 2 weeks. By contrast, in the group of subjects who showed increased NNAL levels at some points during the smoking cessation program, the m7Gua and 8-OHdG levels did not coincide with the smoking cessation duration (Fig. 4).

Correlation between the urinary levels of each biomarker

All data at 0, 2, and 8 weeks from all subjects were used for the correlation analyses. Among the exposure biomarkers, the urinary levels of nicotine, cotinine and NNAL significantly correlated with each other (Table 2). In the cases of the DNA damage biomarkers, the urinary levels of m7Gua were weakly related to the 8-OHdG levels. A comparison of the exposure markers with the DNA damage markers revealed that the urinary levels of cotinine and NNAL positively correlated with the level of m7Gua, but not with the 8-OHdG. The nicotine levels were not correlated with the m7Gua and 8-OHdG levels.

Discussion

In a handful of individuals, the urinary nicotine and cotinine levels remained high after two weeks of the quit-smoking program, even though the median values were low in the results for all participants (Group 1 in Fig. 2a and b). Considering the short biological half-lives of nicotine and cotinine, a reasonable explanation is that
the high levels were caused by smoking before visiting the clinic. Interestingly, among the subjects who completed the quit-smoking program, no one had such high levels of nicotine and cotinine (Group 2 in Fig. 2a and b). These high levels are probably derived from some subjects who could not continue the quit smoking program and had dropped out. Among the subjects who completed the quit-smoking program (Group 2 in Fig. 2), the urinary nicotine and cotinine levels decreased to 1.8 and 3.4% after quitting smoking for 2 weeks, and then to 1.7 and 0.2% after 8 weeks, relative to the values at the beginning of the smoking cessation program. In comparison, the NNAL levels were 31.4 and 12.3% at 2 and 8 weeks. These reduction rates were slower than those in previous reports with subjects in the United States [13, 33]. They may reflect racial differences in the metabolic rates of tobacco-
specific biomarkers. In fact, Asians reportedly had slower rates of nicotine metabolism, as compared with those of Whites and Hispanics [34].

In this study, the smoking status during the quit-smoking program was confirmed by a medical interview of each subject during their visits to the clinic. As a result, 8 subjects mentioned smoking at some point during the quit-smoking program. Among them, however, the nicotine, cotinine, and NNAL levels were increased in only 1 subject. The smoking levels of the other 7 subjects did not elicit the increases in urinary biomarker levels measured during the clinical assessment. In many cases, increased levels of nicotine, cotinine, and NNAL were observed in the subjects who described themselves

**Table 2** Spearman’s rank correlation coefficients of associations between urinary biomarkers of tobacco exposure, 8-OHdG, and m7Gua

| Variable  | Correlation coefficients |
|-----------|--------------------------|
| Nicotine  | Cotinine | NNAL  | m7Gua |
| Cotinine  | 0.80      | –     | –     | –     |
| NNAL      | 0.61      | 0.66  | –     | –     |
| m7Gua     | 0.16      | 0.26  | 0.35  | –     |
| 8-OHdG    | 0.04      | –0.06 | –0.02 | 0.22  |

* indicates p < 0.05. ** indicates p < 0.01
as non-smokers. Considering that these biomarkers are specific for tobacco smoking, some subjects might have not correctly declared their smoking status. In the quitting-smoking program, the subjects visited the clinic at 2 or 4 week intervals. The smoking amounts and durations were not accurately identifiable from the clinical interview. Therefore, it is important to measure the tobacco-specific urinary biomarkers.

Urinary NNAL is a specific biomarker for tobacco exposure. It is not detected in nonsmokers, unless they are exposed to secondhand smoke [15]. Benowitz et al. [35] reported that the average NNAL levels were 183 pg/mg creatinine in the urine for 373 active smokers and 5.19 pg/mg creatinine for 228 passive smokers. In this study, the average NNAL levels of the subjects at the initial visit to the clinic were similar to those of the active smokers in the previous report. For patients who visit the hospitals or clinics every 2 or 4 weeks, NNAL is an appropriate biomarker for evaluating the smoking cessation status. In the group of the subjects with increased NNAL levels at some point, the m’Gua and 8-OHdG levels did not decrease in the treatment period. Smoking during the smoking cessation period may have affected these levels. By eliminating the subjects who showed increased NNAL levels at some points during the smoking cessation program, the following relationships between DNA damage markers and smoking cessation durations have become more apparent.

With regard to urinary DNA damage markers, the decreasing rates of the urinary m’Gua and 8-OHdG were 40.8 and 8.8% after quitting smoking for 2 weeks, and 48.1 and 2.2% after 8 weeks as compared with the beginning of the program. The decreasing rate of the m’Gua was slower than that reported previously (54% reduction after smoking cessation for 1 week) [36]. The reason for this difference may be due to the limited number of subjects and the low m’Gua levels at the beginning of cessation in the previous report. In this study, urinary 8-OHdG levels were decreased by 2 weeks of smoking cessation. However, among the subjects who completed the smoking cessation program, the reduction rate at 8 weeks was limited. Further studies are needed to confirm the sustained effects of the lower 8-OHdG levels elicited by quitting smoking. Several studies have shown reductions of urinary 8-OHdG levels upon quitting smoking [37, 38]. In one study of urinary 8-OHdG levels after quitting smoking, the 8-OHdG levels decreased by 23% at 4 and 26 weeks. There was no change in 8-OHdG levels after 4 weeks of smoking cessation [37], and thus the oxidative stress caused by smoking could be eliminated by quitting smoking for 1 month. The urinary 8-OHdG levels are widely employed as a useful biomarker for monitoring the oxidative stress status involved in cancer induction and lifestyle-related diseases. Although smoking is one of the major factors in elevated 8-OHdG levels, many other factors also increase them, such as ionizing radiation [39], environmental pollutants, lifestyle choices such as alcohol drinking, and so on [22, 40]. Better clarification of the health effects of smoking could be achieved with an assessment combination including another biomarker like m’Gua, which is produced by a different mechanism than that of 8-OHdG.

In a cross-sectional study, the urinary m’Gua levels positively correlated with cigarette smoking [41]. According to Spearman’s rank correlation coefficient (Table 2) in this study, the urinary m’Gua level was weakly associated with the cotinine and NNAL levels as the tobacco exposure markers. The results of our longitudinal epidemiological study were also in good agreement with the previous cross-sectional study [42].

**Conclusions**

In patients participating in a smoking cessation program, the levels of urinary DNA damage markers (m’Gua and 8-OHdG) decreased with the duration of smoking cessation, in the same manner as the smoking exposure markers (nicotine, cotinine and NNAL). The urinary levels of cotinine and NNAL positively correlated with the m’Gua levels. NNAL may be an appropriate exposure marker for evaluating the smoking status of patients in a smoking cessation program.

**Abbreviations**

NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; m’Gua: 7-methylguanine

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**Authors’ contributions**

YK, KK, KN and HY collected the urine samples. YK and KK analyzed nicotine, cotinine and NNAL in urine. Y-S L and YO analyzed 8-OHdG and m’Gua in urine. YK statistically analyzed the data. KK and YK designed and critically discussed the study. All authors read and approved the final manuscript.

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**Availability of data and materials**

Not applicable.

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.
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References
1. Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011;8:13–28.
2. Khanwala SS, Hutsadami D, Hecht SS. Tobacco carcinogen metabolites and DNA adducts as biomarkers in head and neck cancer: potential screening tools and prognostic indicators. Head Neck. 2012;34:441–7.
3. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum. 2004:831–1348.
4. Ministry of Health, Labour and Welfare. National Health and Nutrition Survey. 2018. https://www.mhlw.go.jp/bunya/kenchou/en_kenchou_echuou.html Accessed 10 Jul 2020.
5. Perkins KA, Karelitz JL, Joo NC. Optimal carbon monoxide criteria to confirm 24-hr smoking abstinence. Nicotine Tob Res. 2013;15:978–82.
6. Benowitz NL, Bernert JT, Jacob P, Jarvis MJ, et al. Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. Cancer Res. 1996;56:2546–9.
7. Irie M, Tamae K, Iwamoto-Tanaka N, Kasi H. Occupational and lifestyle factors and urinary 8-hydroxydeoxyguanosine. Cancer Sci. 2005;96:660–6.
8. Watanabe S, Kasai H, Kavi Y. Salivary 8-hydroxydeoxyguanosine as a lifestyle-related oxidative stress biomarker in workers. J Clin Biochem Nutr. 2020;65:67.
9. Kasai H, Kawai K, Yamamoto S, Kondo H, Watanabe S, Ohta M, et al. Measurement of 8-hydroxydeoxyguanosine as an oxidative stress biomarker in saliva by HPLC-CED. Genes Environ. 2018;40:2–5.
10. Cao C, Lai T, Li M, Zhou H, Lv D, Deng Z, et al. Smoking-promoted oxidative DNA damage response is highly correlated to lung carcinogenesis. Oncotarget. 2016;7:18919–26.
11. Kasai H, Svedoba P, Yamazaki S, Kawai K. Simultaneous determination of 8-hydroxydeoxyguanosine, a marker of oxidative stress, and creatinine, a standardization compound, in urine. Ind Health. 2005;43:333–6.
12. Koniewicz ML, Havel CM, Peng MW, Jacob P, Dempsey D, Yu L, et al. Elimination kinetics of the tobacco-specific biomarker and lung cancer 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Cancer Epidemiol Biomark Prev. 2009;18:3421–5.
13. Derby KS, Cottrill K, Caberto C, Carmella SG, Frank AE, Hecht SS, et al. Nicotine metabolism in three ethnic/racial groups with different risks of lung cancer. Cancer Epidemiol Biomark Prev. 2008;17:3526–35.
14. Benowitz N, Koniewicz ML, Eisner M, Lazzano-Ponce E, Zielinska-Danch W, Koszowski B, et al. Urine cotinine underestimates exposure to the tobacco-derived lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in passive compared with active smokers. Cancer Epidemiol Biomark Prev. 2010;19:2795–800.
15. Ichiba M, Matsumoto A, Kondoh T, Horita M, Tomokuni K. Decreasing urinary PAH metabolites and 7-methylguanine after smoking cessation. J Occup Environ Health. 2006;9:754–59.
16. Priemé H, Jorgensen F, Christiansen M, Fullerton RA, Poulsen HE. Effect of smoking cessation on oxidative DNA modification estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine. Cancer Epidemiol Biomark Prev. 2003;12:2392–7.
17. Peterson LA. Context matters: contribution of specific DNA adducts to the genotoxic properties of the tobacco-specific nitrosamine NNK. Chem Res Toxicol. 2017;30:420–33.
18. Rinne ML, He Y, Pachkowski BF, Nakamura J, Kelley MR. N-methylpurine DNA glycosylase overexpression increases alkylation sensitivity by rapidly removing non-toxic 7-methylguanine adducts. Nucleic Acids Res. 2005;33: 2859–67.
19. Suzuki T, Katayama Y, Komatsu Y, Kamiya H. Analysis of large deletion mutations induced by abasic site analog in human cells. Genes Environ. 2018;40:1–8.
20. Rusyn I, Asakura S, Li Y, Kosyk O, Koc H, Nakamura J, et al. Effects of ethylene oxide and ethylene inhalation on DNA adducts, apurinic/apyrimidinic sites and expression of base excision DNA repair genes in rat brain, spleen, and liver. DNA Repair (Amst). 2005;4:1099–110.
21. Czobor PAJ, Harrison K, Shah R, Watson AJ, Agus R, Barber PV, et al. Topographical study of 6-allylnitrosoguanine DNA alkytransferase repair activity and N7-methylguanine levels in resected lung tissue. Chem Biol Interact. 2013;204:98–104.
22. Lee HL, Hsieh YM, Chung CJ, Pu YS, Chang LW, Hsieh DP, et al. Correlation between the urine profile of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolites and N7-methylguanine in ureropath carcinoma patients. Cancer Epidemiol Biomark Prev. 2008;17:3930–5.
23. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res - Rev Mutat Res. 1997;387:147–63.
24. Kasai H, Kawai K. 8-Hydroxydeoxyguanosine, an oxidative DNA and RNA modification. In: Jurgs S, Erdmann WA, Barcziszewski J, editors. Modified nucleic acids in biology and medicine. Cham: Springer International Publishing; 2016. p. 147–85.
25. Kasai H. What causes human cancer? Approaches from the chemistry of DNA damage. Genes Env. 2016;8:19.
26. Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis. 1992;13:2241–7.
27. Arami S, Hirano T, Yamanuchi R, Tomioka Y, Itoh H, Kasai H. Increase of a type of oxidative DNA damage, 8-hydroxydeoxyguanosine, and its repair activity in human leukocytes by cigarette smoking. Cancer Res. 1996;56:2546–9.
28. Irie M, Tamae K, Iwamoto-Tanaka N, Kasi H. Occupational and lifestyle factors and urinary 8-hydroxydeoxyguanosine. Cancer Sci. 2005;96:660–6.
29. Watanabe S, Kasai H, Kawai K. Salivary 8-hydroxydeoxyguanosine as a lifestyle-related oxidative stress biomarker in workers. J Clin Biochem Nutr. 2020;65:67.
30. Kasai H, Kawai K, Li YS, Kawasaki S, Watanabe S, Ohta M, et al. Measurement of 8-hydroxydeoxyguanosine as an oxidative stress biomarker in saliva by HPLC-CED. Genes Environ. 2018;40:2–5.
31. Cao C, Lai T, Li M, Zhou H, Lv D, Deng Z, et al. Smoking-promoted oxidative DNA damage response is highly correlated to lung carcinogenesis. Oncotarget. 2016;7:18919–26.
42. Hu CW, Hsu YW, Chen JL, Tam LM, Chao MR. Direct analysis of
tobacco-specific nitrosamine NNK and its metabolite NNAL in human
urine by LC-MS/MS: evidence of linkage to methylated DNA lesions.
Arch Toxicol. 2014;88:291–9.

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