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Association of hepatitis virus infection, alcohol consumption and plasma vitamin A levels with urinary 8-hydroxydeoxyguanosine in chemical workers

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Abstract

Urinary 8-hydroxydeoxyguanosine (8-OHdG) DNA adduct has been used as a biomarker in epidemiological studies. However, the determinants for urinary 8-OHdG have not been clearly identified. We tested urinary 8-OHdG levels in 205 male workers who had been exposed to vinyl chloride monomer (VCM). Epidemiological information was obtained by an interviewer-administered questionnaire. Hepatitis B surface antigen (HBsAg) and anti-hepatitis C antibody (anti-HCV) were also determined by immunoassay. Plasma antioxidants including Vitamins A and E, α- and β-carotenes were assayed by high performance liquid chromatography. Median of urinary 8-OHdG level was 9.8 ng/mg creatinine (range, 1.4–60.1). Multiple linear regression analysis showed that alcohol drinkers had higher urinary 8-OHdG than those who did not, but there was no dose–response between the amount of alcohol consumption and urinary 8-OHdG. Workers with positive HBsAg, anti-HCV and elevated plasma Vitamin A level were independently associated with higher levels of urinary 8-OHdG, whereas age, smoking, body mass index, plasma α- and β-carotenes, Vitamin E levels, or VCM exposure did not show such an association. The results suggest that active inflammation of hepatitis B and C, alcohol consumption and higher Vitamin A level can induce oxidative stress. Thus, we conclude that potential determinants need to be considered in epidemiological studies when urinary 8-OHdG is used as a biomarker.

Keywords: 8-OHdG; Hepatitis virus infection; Micronutrient; Alcohol drinking; Smoking

1. Introduction

Oxidative damage in DNA can be caused by a variety of exposures to endogenous and exogenous compounds [1]. This type of damage, if not repaired, can lead to genetic mutation [2]. 8-Hydroxydeo-

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xyguanosine (8-OHdG) is one of the most abundant oxidative DNA adducts. It is mutagenic in nature and can cause G to T transversion [3].

The repair process for 8-OHdG inflicted damage results in excised 8-OHdG adduct being excreted into the urine [2,4]. Because of easy collection, urinary 8-OHdG has been used as a biomarker in occupational epidemiological studies [5]. Age, gender, smoking and the use of dietary supplements have been reported to be associated with urinary 8-OHdG [1,5]. However, the association between non-occupational factors and urinary 8-OHdG has not been consistent [5]. We have used a comprehensive design to determine the role of occupational and non-occupational factors in the generation of urinary 8-OHdG in a group of workers exposed to vinyl chloride monomer (VCM).

2. Materials and methods

2.1. Study subjects

A total of 205 males with greater than 6 months of VCM exposure from five polyvinyl chloride (PVC) production plants were included in the analysis. Information on their smoking, alcohol consumption, medication practices, and their detailed occupational histories were collected by interviewer-administered questionnaires with the workers' consent. Because the usual alcohol consumption in the study subjects was relative low, habitual alcohol drinking was defined as alcoholic consumption of at least once a week and more than 80 g of alcohol per week. Additionally, in this report, overweight was evaluated by body mass index (BMI), which was defined as kilograms per m^2. VCM exposure levels for these study subjects were based on our previously published work [6]. Urine and venous blood were collected in subjects with fasting during medical surveillance, then stored at 4 °C and processed on the same day. Venous blood was further separated into plasma, buffy coat and red blood cells. All specimens were stored under −80 °C until analysis.

2.2. Determination of urinary 8-OHdG level

Urine samples were centrifuged at 2000 *g* for 10 min and the supernatants were used for the determination of 8-OHdG levels with a competitive ELISA kit (Japan Institute for the Control of Aging, Japan). The determination range was 0.5–200 ng/ml. The 8-OHdG monoclonal antibody and urine sample were loaded at 50 μl on a microtiter plate which has been coated with 8-OHdG, and incubated at 37 °C for 1 h, in accordance with the instructions of the manufacturer. After washing, the antibodies that remained bound to the 8-OHdG in the sample were further bound with the horseradish peroxidase-conjugated secondary antibody. Subsequent addition of 3,3′,5,5′-tetramethylbenzidine resulted in the development of color intensity proportional to the amount of antibody bound to the plate. The color reaction was terminated by stop solution (phosphoric acid) and the absorbance was measured using a spectrophotometric plate reader at 450 nm wavelength. Urinary creatinine was determined with a Hitachi 7050 autoanalyser at the Department of Laboratory Medicine in the National Taiwan University Hospital (NTUH). Finally, urinary 8-OHdG levels were adjusted by urinary creatinine levels.

2.3. Determination of plasma micronutrients levels

Micronutrients levels for Vitamins A, E and α- and β-carotene were measured by high-performance liquid chromatography (HPLC, Hitachi D6000) using the methods described by Miller et al. in 1984 [7]. Vitamin A level was monitored by the ultraviolet detector with a 325 nm filter. α-and β-Carotenes were measured using the visible detector with a 466 nm filter and Vitamin E was monitored by the UV detector with a 280 nm filter. Total equilibration time between runs was approximately 14 min. The correlation coefficients of standard curve were all greater than 0.990. The recovery rates ranged from 80 to 120%, and coefficients of variation (CV) for reproducibility were all less than 10%. Because Vitamin E circulates in the blood-associated lipids, plasma concentration of Vitamin E was adjusted for blood lipids by diving by the sum of plasma cholesterol and triglyceride concentrations [8].

2.4. Determination of HBsAg and anti-HCV

Hepatitis B virus surface antigen (HBsAg) and anti-hepatitis C virus antibody (anti-HCV) were

assayed with radioimmunoassay (RIA, Abbott Lab, Chicago IL, USA) and enzyme-linked immumosorbent assay (ELISA, Austria-II, Abbott Lab, Chicago IL, USA), respectively, at the Department of Laboratory Medicine, NTUH.

2.5. Statistical analysis

Non-parametric test was used to test the difference of 8-OHdG in each variable. Subsequently, multiple linear regression analysis was conducted to examine the association of urinary 8-OHdG levels with various variables including age (<40 and ≥40), VCM exposure (<1, 1–4 and ≥5 ppm), smoking (no, <10 cigarettes per day and ≥10 cigarettes per day), alcohol consumption (no, <140 g per day, ≥140 g per day), BMI (<25 and ≥25), HBsAg (positive and negative) and anti-HCV (positive and negative), and plasma vitamins (μg/ml for Vitamins A and E and β-carotene and μg/dl for α-carotene). Mean of age and alcohol consumption were used as cut-off points. A cut-off point of 25 kg/m^2 was used for BMI, which had been recommended by World Health Organization to define overweight. Regression coefficient and their standard error of means were also computed.

3. Results

The basic characteristics of the study subjects are presented in Table 1. The average age was 39.7 years. Smokers accounted for 41.0% of the study population, and alcohol consumption 19.5%. HBsAg was positive in 18.0% of workers and anti-HCV in 2.0%. Median plasma level was 0.58 μg/ml for Vitamin A, 4.98 μg/dl for α-carotene, 0.19 μg/ml for β-carotene and 7.28 μg/ml for Vitamin E. Median urinary 8-OHdG level for the study subjects was 9.8 ng/mg creatinine (range, 1.4–60.1, Table 2). Subjects greater than 40 years old had higher 8-OHdG level than those less than 40 years old. Those smoking over 10 cigarettes per day had higher 8-OHdG level than subjects who never smoked or smoked less than 10 cigarettes per day. Workers with BMI less than 25 also had higher 8-OHdG than those with BMI higher than 25. However, the association for age, cigarette smoking and BMI was not significant.

Table 1
Basic characteristics of study subjects

Variables	$N = 205$ (%)
Age (year)	
<40	96 (46.8)
≥40	109 (53.2)
VCM exposure	
<1 (ppm)	79 (38.5)
1–4 (ppm)	88 (42.9)
≥5 (ppm)	38 (18.6)
Smoking	
No	121 (59.0)
<10 cigarettes per day	13 (6.3)
10–19 cigarettes per day	71 (34.7)
Alcohol drinking	
No	165 (80.5)
<140 g per day	24 (11.7)
≥140 g per day	16 (7.8)
BMI	
<25	131 (63.9)
≥25	74 (36.1)
HBsAg	
Negative	168 (82.0)
Positive	37 (18.0)
Anti-HCV	
Negative	201 (98.0)
Positive	4 (2.0)
Antioxidants	
Vitamin A (μg/ml)	0.58 (0.09–2.02)[a]
α-Carotene (μg/dl)	4.98 (0–46.37)
β-Carotene (μg/ml)	0.19 (0–0.93)
Vitamin E (μg/ml)	7.28 (2.11–23.4)

[a] Median (range).

Workers with VCM exposure greater than 5 ppm also had higher 8-OHdG than those with exposure less than 5 ppm ($P = 0.09$). Those who consumed alcohol with total amount either greater than or less than 140 g per week had higher urinary 8-OHdG than those who did not ($P < 0.05$). However, there was no dose–response between amount of alcohol consumption and urinary 8-OHdG.

Workers with positive HBsAg also had higher urinary 8-OHdG than those without ($P < 0.05$). The difference in urinary 8-OHdG was even more prominent between those with anti-HCV and those without ($P < 0.01$). Plasma Vitamin A level was also found to be positively associated with urinary 8-OHdG, whereas

Table 2
Urinary 8-OHdG in ng/mg creatinine stratified by different variables

Variables	*N*	Mean ± S.D.	Median (range)
Total	205	12.7 ± 9.2	9.8 (1.4–60.1)
Age (year)			
<40	96	12.6 ± 10.7	9.3 (1.4–60.1)
≥40	109	12.9 ± 7.6	10.8 (2.2–45.9)
VCM exposure			
<1 (ppm)	79	12.6 ± 9.4	9.8 (2.2–56.7)
1–4 (ppm)	88	11.7 ± 8.4	9.6 (1.4–45.9)
≥5 (ppm)	38	14.7 ± 10.7	11.8 (3.9–60.1)
Smoking			
No	121	11.5 ± 7.1	9.5 (1.4–45.9)
<10 cigarettes per day	13	14.3 ± 15.7	6.7 (4.8–60.1)
10–19 cigarettes per day	71	13.9 ± 10.8	10.6 (3.3–56.7)
Alcohol drinking			
No	165	11.4 ± 6.8	9.4 (1.4–45.9)
<140 g per day[a]	24	18.1 ± 16.5	13.0 (3.0–60.1)*
≥140 g per day	16	17.3 ± 11.6	14.0 (3.0–39.0)*
BMI			
<25	131	13.4 ± 10.0	10.5 (1.4–60.1)
≥25	74	11.4 ± 7.5	9.2 (3.7–54.5)
HBsAg			
Negative	168	12.3 ± 9.5	9.2 (1.4–60.1)*
Positive	37	14.7 ± 7.4	13.8 (3.0–32.3)
Anti-HCV			
Negative	201	12.4 ± 8.8	9.7 (1.4–60.1)**
Positive	4	28.1 ±16.6	26.6 (13.2–45.9)
Vitamin A (μg/ml)			
<0.61[a]	101	11.7 ± 8.4	9.5 (1.4–54.4)[b]
≥0.61	104	13.6 ± 10.1	10.2 (3.8–60.1)
α-Carotene (μg/dl)			
<6.1[a]	123	13.3 ± 9.7	10.1 (3.0–56.7)
≥6.1	82	11.4 ± 8.6	9.2 (1.4–60.1)
β-Carotene (μg/ml)			
<0.23[a]	125	12.8 ± 9.4	9.6 (1.4–56.7)
≥0.23	80	12.3 ± 9.3	9.8 (2.2–60.1)
Vitamin E (μg/ml)[c]			
<8.0[a]	119	12.7 ± 10.3	9.4 (1.4–60.1)
≥8.0	86	12.4 ± 7.6	10.4 (3.0–45.9)

[a] Mean.
[b] $P = 0.06$.
[c] Adjusted for blood lipids.
* $0.01 < P < 0.05$.
** $P < 0.01$.

Table 3
Regression coefficient (in ng/mg creatinine) and their standard errors (S.E.) from constructing multiple regression model for urinary 8-OHdG

Variables	Regression coefficient	S.E.	*P*-value
Intercept	7.2	3.0	<0.05
Age			
<40 year vs. ≥40 year	0.5	1.4	0.82
VCM exposure			
1–4 ppm vs. <1 ppm	−1.6	1.5	0.3
≥5 ppm vs. <1 ppm	0.01	2.0	0.9
Smoking			
<10 cigarettes per day vs. no	0.4	1.6	0.8
≥10 cigarettes per day vs. no	−0.3	2.2	0.9
Alcohol drinking			
<140 g per day vs. no	6.3	2.4	<0.01
≥140 g per day vs. no	6.1	2.4	0.02
BMI			
≥25 vs. <25	−1.5	1.4	0.53
HBsAg			
Positive vs. negative	3.2	1.7	0.06
Anti-HCV			
Positive vs. negative	17.8	5.7	<0.01
Antioxidants			
Vitamin A (μg/ml)	7.2	3.5	0.03
α-Carotene (μg/dl)	0.2	0.1	0.28
β-Carotene (μg/ml)	−2.0	5.6	0.72
Vitamin E (μg/ml)[a]	−0.2	0.2	0.39

[a] Adjusted for blood lipids.

plasma α- and β-carotenes and Vitamin E levels were not associated with urinary 8-OHdG.

In the subsequent multiple regression analysis (Table 3), positive HBsAg ($P = 0.06$), positive anti-HCV ($P < 0.01$), increased alcohol consumption ($P < 0.01$ for those having alcohol consumption but less than 140 g per week, $P = 0.02$ for those having alcohol consumption but greater than 140 g per week) and increased plasma Vitamin A levels ($P = 0.03$) were associated with increased 8-OHdG in urine. However, age, VCM exposure, smoking and BMI were not associated with urinary 8-OHdG. Analysis also showed that plasma levels of α- and β-carotenes and adjusted Vitamin E were not associated with 8-OHdG.

4. Discussions

The results revealed that active inflammation of hepatitis B and C, alcohol consumption, and increased plasma Vitamin A levels were associated with urinary 8-OHdG; however age, smoking, BMI, and VCM exposure were not associated with urinary 8-OHdG.

Inflammation of hepatocytes due to hepatitis B and C has been reported to be associated with increased reactive oxygen species (ROS) in the liver tissue [9,10]. Here, we have demonstrated that increased ROS by HBV and HCV infection can be detected in urine. Thus, HBV and HCV infection should be considered in studies of oxidative damage, particularly in areas with high prevalence of hepatitis B and C infection.

It is interesting to observe that alcohol consumption was associated with 8-OHdG. This kind of association has not been reported in previous occupational studies [5], although alcohol consumption has been reported to generate ROS presumably through the effect of cytochrome p450 2E1 [11,12]. However, the dose–response was not observed in our study. This was probably due to a small range in the level of alcohol consumption. More studies are needed to corroborate the effect of alcohol consumption on urinary 8-OHdG.

VCM is a known mutagen and has been associated with increased genotoxicity in human studies [13–15]. Recent studies also revealed that VCM exposed workers had increased DNA single strand breaks [6,16], which suggest that oxidative damage may play a role in VCM-related DNA damage. Our study also revealed that urinary 8-OHdG increased in workers with VCM exposure greater than 5 ppm, albeit less significantly. Because the air level of VCM in the work sites is relatively low, a better assessment of individual VCM exposure may increase the sensitivity in detecting the difference of 8-OHdG.

Although those smoked greater than 10 cigarettes per day had higher urinary 8-OHdG than non-smokers, the relationship was not significant. Since cigarette smoke contains ROS, the association between cigarette smoking and urinary 8-OHdG has been previously reported [3]. However, the inconsistent findings have been documented in many previous occupational studies [5]. Oxidative damage occurs rapidly following exposure, and this damage can be repaired rapidly. Since onsite workers were not allowed to smoke regularly during the working hours for the sake of safety, the effects of smoking on 8-OHdG in workers are less likely to appear in the model.

Loft et al. [1] reported that overweight persons had a lower metabolic rate than lean ones. Our study also revealed an inverse association between BMI and 8-OHdG. However, the association was not significant, probably most of our workers were not obese as the average of BMI was 24.1.

Antioxidants have been known to reduce oxidative damage in cultured cells, animals and humans [17–19]. Decreased levels of 8-OHdG have been demonstrated in those with increased intake of antioxidants-rich vegetable and fruits [20]. However, the exact effects of antioxidants on mutagenesis and carcinogenesis remain unclear. Opposite findings have reported that increased risk of lung cancer can develop in smokers who receive antioxidants [21,22]. Excess amounts of Vitamin A have been found to increase chromosomal aberration in cultured lymphocytes [23]. Further, rats treated with retinol also experience higher incidence of pheochromocytomas [24]. Thus, antioxidants as redox agents may have prooxidant effects [25]. Our results reveal that increased levels of Vitamin A are positively associated with increased 8-OHdG. It appears antioxidants may involve a complex mechanism in carcinogenesis.

It is clear that active inflammation of hepatitis B and C is associated with increased generation of ROS and subsequent 8-OHdG. However, the lower consistency between 8-OHdG and potential confounders underscores that oxidative stress may be involved in a very complex process and manifested in a variety of ways. Before the exact role of 8-OHdG is clearly examined, potential determinants including cigarette smoking, alcohol consumption, hepatitis virus infection, BMI, and dietary habits need to be considered in future epidemiological studies when urinary 8-OHdG is used as a biomarker.

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