

# Urinary 8-Hydroxy-Deoxyguanosine as a Biomarker of Oxidative DNA Damage in Employees of Subway System

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**Abstract-** Exposure to air pollutants, steel dust or other occupational and environmental hazards as oxidative stress have adverse effects on subway workers' health. Oxidative stress generates an excessive amount of reactive oxygen species (ROS) and Oxygen Free Radicals during their work time in the tunnels. Once DNA is repaired, Urinary 8-hydroxy-deoxyguanosine (8-OHdG) is excreted in the urine. Therefore, urinary level of 8-OHdG can reflect the extent of oxidative DNA damage. The aim of this study was to document the oxidative stress caused by exposure to these hazards by measuring 8-OHdG in workers urine. We collected urine samples of 81 male subway workers after their working shift. The concentration of urinary 8-OHdG was measured by ELISA method. We used linear regression analysis to compare the level of urinary 8-OHdG as a biomarker of oxidative stress between workers in tunnels and other staff. The mean concentration of urinary 8-OHdG for workers in the tunnel was 58.05 (SD=28.83) ng/mg creatinine and for another staff was 54.16 (SD =26.98) ng/mg creatinine. After adjustment for age, smoking, driving and a second job in a linear regression model, the concentration of 8-OHdG for the exposed group was significantly higher than unexposed group ( $P=0.038$ ). These findings confirm that the concentration of urinary 8-OHdG for workers who work in tunnels was significantly higher than the other staff. Additional investigations should be performed to understand that which ones of occupational exposures are more important to cause oxidative stress.

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**Keywords:** Deoxyguanosine; DNA damage; Occupational exposure; Subway system

## Introduction

Reactive oxygen species (ROS) and Oxygen Free Radicals are endogenously generated as a result of metabolic reactions in human organisms (1). However, exposure to air pollutants, steel dust or other occupational and environmental hazards as oxidative stress can generate an excessive amount of ROS (2). Within tissues, DNA is repeatedly being damaged by ROS (3-4) and oxidative damage to DNA by ROS is known to be one of the most important mechanisms for the pathogenesis of cancer, cardiovascular and other chronic diseases (5).

It is shown that subway workers may develop various diseases and injuries (6). The prevalence of gastrointestinal and cardiovascular disease among subway train drivers has also been reported to be high, but the major health concern was cardiovascular diseases including hypertension, myocardial infarction

and stroke (6-7). In addition, lung and bladder cancer, post-traumatic stress disorders and musculoskeletal disorders are considerably more common among transit workers as well (6).

Transit workers who work underground in specific conditions and are considerably exposed to air pollution with unfavorable microclimate and steel dust including Iron (Fe), Manganese (Mn), and Chromium (Cr) (5). Seaton *et al.*, indicated that drivers and station staff would have maximum exposures 200  $\mu\text{g}/\text{m}^3$  PM 2.5 (Particulate matter) over 8 hours in London Underground (8). The TEACH (Toxic Exposure Assessment, a Columbia/Harvard) study showed that the concentration of steel dusts in New York City subway was 100 times more than home indoor and outdoor setting (9).

There are some debates that air pollutants and other occupational hazards can induce producing excessive ROS within tissues during work-related activities in

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tunnels. Few studies are conducted regarding adverse effects of occupational exposures in underground subway workers, and there is increasing concern about it as well.

Recently, 8-OHdG is known as a biomarker of oxidative stress. Once DNA is repaired, 8-OHdG is excreted in the urine. Therefore, urinary level of 8-OHdG can reflect the extent of oxidative DNA damage (10-12).

There are many studies investigating concentration of 8-OHdG as an anticipating factor for cancers and degenerative diseases (13). Also, Pliger *et al.*, in 2006 has conducted a review of oxidative DNA damage induced by different occupational exposures and 8-OHdG serves as a potential biomarker for detection of DNA damage (5). This study demonstrates that whether hazards of working in tunnels including chemical and physical hazards could cause elevated urinary 8-OHdG level as a biomarker of DNA damage or not.

## Materials and Methods

From September to October 2012, 89 applicants from Tehran subway system were randomly selected. Inclusion criteria were male sex, normal Body Mass Index (BMI) (19-24 kg/m<sup>2</sup>), and more than one year employment history in current job task. Applicants were excluded if they refused to provide urine sample, suffered from acute or chronic diseases (e.g. cancer or any other known disease) or had taken medicine during the past seven days prior to taking the sample. Participants were interviewed and samples after working shift for shift workers and after work hours in office workers were collected.

A trained researcher interviewed all participants. They provided information via data collection sheets regarding demographic characteristics including age, degree of education (university degree vs. high school and lower level) and marriage status (married vs. single, where divorced and widowed individuals were considered as single).

They were asked about their lifestyle choices including smoking (tobacco or other types of substance) where ex-smokers were considered as non-smoker, drinking alcohol, consumption of vitamin and doing exercise (at least twice a week for both).

For job-related variables, we asked about years of work experience, job satisfaction, shift working, and having a second job. Job satisfaction was determined based on the visual analog scale (VAS) from 1- 10. The exact nature of job responsibilities and work environment were recorded. Drivers and passenger

service providers (ticket sellers, collectors, and workers at the platforms) worked in tunnels. The executives, officers, administrators, and clerks worked outside of tunnels.

All participants obtained informed consent through the survey and medical ethics committee of Tehran University approved this research protocol. Each participant was asked to sign a written consent form.

Urine samples were transported to the laboratory in a cold box (0°C) and stored at -80 °C for further analysis. Urinary 8-OHdG concentration was measured using competitive enzyme-linked immunosorbent assay (CAYMAN 8-OHdG EIA Kit, USA). Briefly, frozen urine samples were thawed at room temperature and centrifuged at 2000g m/s<sup>2</sup> for 10 min. to remove the particulate matters. Samples were then diluted with water (1:100 (V/V)) and the antibody and tracer was added to the aliquot of each sample or standard sample in microtiter plates (1:1 (V/V)) pre-coated with 8-OHdG and incubated for 18 h at 4°C. The plates were then washed 5 times. Ellman's reagent was added to each well (200 µl) and the plates were covered and incubated for 120 min. while gently being shaken. The intensity of the color produced in each well was measured at the wavelength of 405 nm using a computer-controlled microplate reader (ELISA reader, Stat Fax, USA). The urinary creatinine level was determined by an automated analyzer (Technicon RA-1000, USA) and expressed as ng/dl. Finally, the urinary 8-OHdG concentration was reported as ng/mg of creatinine.

We described the "quantitative variables" by mean, standard deviation, and range, and the "qualitative variables" as frequencies and in percentile units. Then we considered working in tunnels as "exposure", and workers in tunnels as the "exposed group" and the rest as "unexposed group". We compared independent variables between exposed and non-exposed groups. For the quantitative variables, we used independent t-test or Mann-Whitney U test, and for the qualitative variables we used chi-square test.

To find the relation between 8-OHdG as the dependent variable and our independent variables in a univariate analysis, we used Spearman's correlation test for quantitative variables and Mann-Whitney U test for binomial variables. A linear regression analysis was used to assess association between level of 8-OHdG as dependent variable and working in tunnel as independent variable or predictor factor after adjustment for potential confounding factors (i.e. age, smoking, having second job, and working as a driver). The level

of significance for all analyzes was 0.05.

## Results

The study population consisted of 89 subway male employees. Eight participants were excluded from the

study: four participants had a cold, two had a history of asthma and took medicine, and one was hypothyroid and one vegetarian.

The characteristics were compared with exposed and control group (Table 1).

**Table 1. Characteristics of the study population**

Characteristics		Total (N=81)	Exposed group (N=38)	Unexposed group (n=43)	P- value
		N(%)	N(%)	N(%)	
<b>Age</b>	(year) (mean± SD)	32.63±7.37	29.29±3.61	35.58±8.55	0.001
<b>Work experience</b>	(year) (mean± SD)	8.49±7.09	4.76±3.94	11.79±7.63	0.001
<b>Marriage status</b>					
	Single	23(28)	16(42)	7(16)	0.01
	Married	58(72)	22(58)	36(84)	
<b>Education</b>					
	High school	20(25)	3(8)	17(40)	0.001
	University degree	61(75)	35(92)	26(60)	
<b>Smoking</b>					
	No	60(74)	26(68)	34(79)	0.31
	Yes	21(26)	12(32)	9(21)	
<b>Shift work</b>					
	No	13(16)	0(0)	13(30)	0.001 <sup>[1]</sup>
	Yes	68(84)	38(100)	30(70)	
<b>Vitamin Usage</b>					
	No	61(75)	25(66)	36(84)	0.062
	Yes	20(25)	13(34)	7(16)	
<b>Exercise</b>					
	No	38(47)	17(45)	21(49)	0.61
	Yes	43(53)	21(55)	22(51)	
<b>Job satisfaction</b>					
	Low	31(38)	11(29)	20(46)	0.1
	High	50(62)	27(71)	23(54)	
<b>Second job</b>					
	No	73(58)	36(49)	37(51)	0.3
	Yes	8(39)	2(25)	6(75)	

<sup>1</sup>Fisher test

The participants in the exposed group were younger and had a higher level of education ( $P=0.001$ ).

The smoking prevalence was lower among the staff who worked outside the tunnel than workers in the tunnels ( $P=0.02$ ).

Shift working was more frequent among workers in the tunnels. The average length of employment was shorter among workers in the tunnels 4.7 (SD=3.9) than the other staff 11.7 (SD=7.6).

No significant difference was found between workers in the tunnel and the other applicants with respect to the prevalence of consumption of vitamin supplements ( $P=0.06$ ). For job satisfaction level, based on VAS, no significant differences were found ( $P=0.10$ ).

The mean concentration of urinary 8-OHdG for

workers in the tunnel was 58.05 (SD=28.83) ng /mg creatinine and for another staff was 54.16 (SD=26.98) ng/mg creatinine. After adjustment for age, smoking, driving and a second job in a linear regression model concentration of 8-OHdG for the exposed group was significantly higher than unexposed group ( $P=0.038$ ). Results are presented in Table 2.

**Table 1. General linear regression of 8-OHdGs level with potential confounders (n=81)**

	B	Std. Error	Sig.
<b>Working in the tunnels</b>	41.47	19.61	0.038
<b>Age</b>	0.281	0.459	0.54
<b>Smoking</b>	-12.73	6.96	0.072
<b>Second job</b>	-17.41	10.21	0.092
<b>Driving</b>	-37.41	19.63	0.059
<b>Constant</b>	51.00	16096	0.004

## Discussion

Present findings indicate that working in the tunnels can cause elevated urinary 8-OHdG in Tehran subway system employees in comparison with workers out of tunnels.

There is a great deal of interest in determining whether the increase in prevalence of mentioned diseases can be accounted for by high airborne particle concentrations, high occupational stresses, exposure to magnetic field or any hazards of working in the tunnels.

This study compared the urinary levels of 8-OHdG (suggesting repair of DNA after oxidative damage) between Iranian subway system male employees who work in the tunnels and those who work outside the tunnels.

We decided to measure 8-OHdG level in the urine as an indicator of oxidative DNA damage because this method is non-invasive, production of artifacts during sampling is rare, and 8-OHdG is very stable in the urine (14). Previous studies have reported that 8-OHdG level is affected by age, BMI and diseases (15-16) and content of this biomarker in urine is higher in smokers (17).

Alcohol drinking and vitamin or energy drink consumption is positively associated with 8-OHdG excretion in urine (18). We adjusted current results for all above mentioned confounders.

In this study, some participants had secondary jobs. To eliminate this confounding effect, we adjusted for a secondary job in the linear regression model as well.

Since it is unlawful to drink alcohol in Iran, people tend to hide their alcohol consumption. One limitation of current study, therefore, was that the potential effect of alcohol consumption in the subjects could not be accounted for. Another limitation was the unavailability of the level of PM 2.5 and the precise amount of any steel dust in the tunnels.

Based on previous studies 8-OHdG is affected by nutrition. In this study, however, it was not feasible to account for this confounder. Nevertheless, subway workers; in particular those working in shifts such as drivers and passenger service officers; often have a meal in the workplace. As a result of having the same socioeconomic conditions, they tend to maintain a relatively similar diet.

Despite considerable effort to standardize the measurement of this biomarker in urine, the result depends on the method used and as a consequence, there is inter-laboratory variation in basic 8-OHdG level (19).

In respect to different DNA repair capacity between people, the level of this biomarker cannot reflect the

exposure to environmental and occupational hazards (20).

One of the most important hazards of working in the tunnels is air pollution, in particular, exposure to steel dusts. Several studies have assessed to show elevated particle levels in underground subway systems (PM 2.5). The London and Stockholm subway systems are reported to be very elevated in PM 2.5 levels in the hundreds of micrograms per meter cube. Also, transit workers are expected to have appreciably higher enhanced exposure to Iron (Fe), manganese (Mn), and Chromium (Cr) (21-22). Elevated Fe level has also been reported for the subway system in London, Stockholm, Washington DC, and Tokyo (23-25). Previous studies showed that increasing level of PM 2.5 has a strong relation with urinary excretion of 8-OHdG among workers (2). Kim *et al.*, found that time weighted average PM 2.5 in 8 hours concentration, and urinary 8-OHdG has significant correlation (2).

Although measurement of 8-OHdG has been used in clarifying the extent of occupational and environmental exposures in many studies, no consensus has been reached for a dose-response relation between level of exposures and urinary 8-OHdG level (5).

Exposure to traffic exhaust could be one of the most important factors for DNA damage as well (26). Chuang *et al.*, in 2003 showed that because of increasing exposure to traffic exhaust, level of 8-OHdG in taxi drivers was significantly higher than community men. For long distance bus drivers, this biomarker has increased compared to the office workers as well (27).

In addition to traffic exhaust, Subway drivers are subject to a considerable level of work-related stress and the other hazards including air pollution on a daily basis. It is appreciated that the drivers have the most mentally demanding task among other employees in the subway system as the main burden of caring for people's lives fall on the drivers. However, the purpose of this study was merely to investigate the occupational hazards of working in the tunnel environment. Therefore, we adjusted for driving as a confounder to ensure that the concentration of 8-OHdG in urine was mainly reflective of working in tunnel conditions. The results of this study suggest that working in the tunnels could potentially be an even more potent predictor of increasing 8-OHdG level than driving.

The results showed that working in the tunnels clearly correlated with higher urinary 8-OHdG excretion. As mentioned above, people working in tunnels are exposed to many different hazards including occupational stress, shift working, air pollution, and exposure to the magnetic field. However, it is unclear

which hazard has the largest effect. To design and propose effective programs to eliminate or decrease the adverse effects, it is crucial to establish the contribution of each one of above mentioned occupational hazards on the employee's health independently. To this end, more comprehensive studies will be required.

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