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ORIGINAL ARTICLE

Traffic air pollution and oxidatively generated DNA damage: can urinary 8-oxo-7,8-dihydro-2-deoxiguanosine be considered a good biomarker? A meta-analysis

Daniele Lettieri Barbato[1,3], Gianfranco Tomei[2], Francesco Tomei[3], and Angela Sancini[3]

[1]*Food and Nutrition Unit, IRCCS San Raffaele Pisana, Rome, Italy and Departments of* [2]*Psychiatric Science and Psychological Medicine, and* [3]*Occupational Medicine, University of Rome 'Sapienza,' Rome, Italy*

Abstract
Oxidative stress is one of the mechanisms through which traffic-related air pollution causes adverse effects on human health. The urinary excretion of 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) has often been used as a biomarker to evaluate the effect of air pollution on subjects occupationally exposed. We used a meta-analysis to evaluate the effect of traffic air pollution on urinary 8oxodG levels in healthy workers. We observed higher urinary 8oxodG levels in non-smoking exposed subjects compared with smokers. This difference was clearer when an HPLC assay was used. These results show that urinary 8oxodG can be used as a biomarker to evaluate the pro-oxidant effects of vehicle exhaust emissions on DNA in exposed workers.

Keywords: *Oxidative stress; environmental pollution/ecotoxicology; DNA adducts/repair mechanisms*

Introduction

Epidemiological studies conducted in metropolitan areas have demonstrated that exposure to traffic air pollution is associated with increases in mortality, even if the mechanism remains unclear (Brunekreef 2007, Bräuner et al. 2007). In recent years a growing body of evidence has attributed to vehicle exhaust emissions the ability to modulate the body's redox system through an increase of pro-oxidant species and a decrease of antioxidant molecules (Yang & Omaye 2009). This condition, defined as oxidative stress, is linked to several DNA lesions, including modified bases, which are considered a potential cause of cancer (Loeb 2001).

Among the biomarkers of oxidatively damaged DNA, urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) has received great interest (Cooke et al. 2009a). Urinary 8oxodG is thought to be produced mainly by interactions of hydroxyl radicals, and possibly singlet oxygen and one-electron oxidants, with host 2′-deoxyguanosine 5′-triphosphates in the 2′-deoxyribonucleotide pools. Once introduced into DNA, 8oxodG can exert mutagenic effects on the host and thus it must be rapidly removed from the body. This is attained by a complicated process of repairing mechanisms, targeted toward both DNA and the 2′-deoxyribonucleotide pools, which result in the excretion of stable products via urine, thus providing a non-invasive assessment of oxidative stress (Garratt et al. 2010). Although several different techniques are available for measuring urinary 8oxodG levels, high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assays (ELISAs) are the most reliable methods (Cooke 2009b). However, there is a significant discrepancy between the chromatographic and immunoassay approaches, and intratechnique agreement among all available chromatography-based assays and ELISAs is yet to be established (Cooke et al. 2010, Evans et al. 2010).

In an occupational setting, research has consistently identified early biomarkers of exposure to the traffic air pollution. However, from literature analysis we have contrasting data about the impact of traffic air

Address for Correspondence: Francesco Tomei, Department of Occupational Medicine, University of Rome 'Sapienza,' Viale Regina Elena 336, Rome, 00161 Italy. E-mail: francesco.tomei@uniroma1.it

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pollution on urinary 8oxodG levels in exposed workers (Singh et al. 2008, Rossner et al. 2007, Chuang et al. 2003). In occupational studies, cigarette smoke has been identified as an important confounding factor that misleads urinary 8oxodG levels in workers exposed to traffic air pollution (Mizoue et al. 2006, Harri et al. 2005).

To examine the impact the traffic air pollution in an occupational setting, we undertook a systematic review of all published case-controlled studies that measured urinary 8oxodG levels in exposed workers. This effect on urinary 8oxodG concentration was evaluated by stratifying the subjects in relation to smoking or non-smoking habits and in relation to analytical methods used in each study. A meta-analysis statistical method was used to achieve our aims.

Methods

Study selection

All papers published in scientific journals from1985 to February 2010, were examined for inclusion in the meta-analysis. Papers suitable for inclusion were identified by a systematic research in the following databases: PubMed, EMBASE and Toxline. All article references were examined in order to find further papers useful for the meta-analysis. We considered studies in any language that were published as full articles.

To be included in our meta-analysis, all papers had to meet the following inclusion criteria: (1) case–control studies; (2) healthy and normal-weight workers daily exposed to vehicle exhaust emissions; (3) urinary levels of 8oxodG as biomarkers of oxidatively generated DNA damage (tested with an ELISA or HPLC); (4) statistical parameters reported as mean, standard deviations (SD) or standard error (SE) or 95% confidence intervals (CIs) of the urinary 8oxodG levels in exposed and not-exposed workers. Studies were excluded if only abstracts were published or information on subjects and biomarkers was incomplete.

This systematic research detected 468 potentially relevant publications. A first titles screening led to 19 potentially relevant studies, but after full-text reading, four studies were excluded because they did not consider subjects professionally exposed to traffic air pollution (Mukherjee et al. 2004, Kim et al. 2004, Chen et al. 2007, Buthbumrung et al. 2008), three studies were excluded because parts of the data were absent (Wei et al. 2009, Abder-Rhman & Nusair 2007, Nilsson et al. 1996), one study was excluded because it considered workers with disease (Allen et al. 2009) and another because there was no control group (Lagorio et al. 1994). Moreover, two studies were excluded because they analysed 8oxodG in peripheral blood mononuclear cells (Ayi Fanou et al. 2006, Singh et al. 2007) (Figure 1).

Eight studies were chosen for this systematic literature analysis; their main features are shown in Table 1.

Data extraction and quality assessment

A form to collect data and quality characteristics was designed and tested. Two authors (D.L.B. and G.T.)

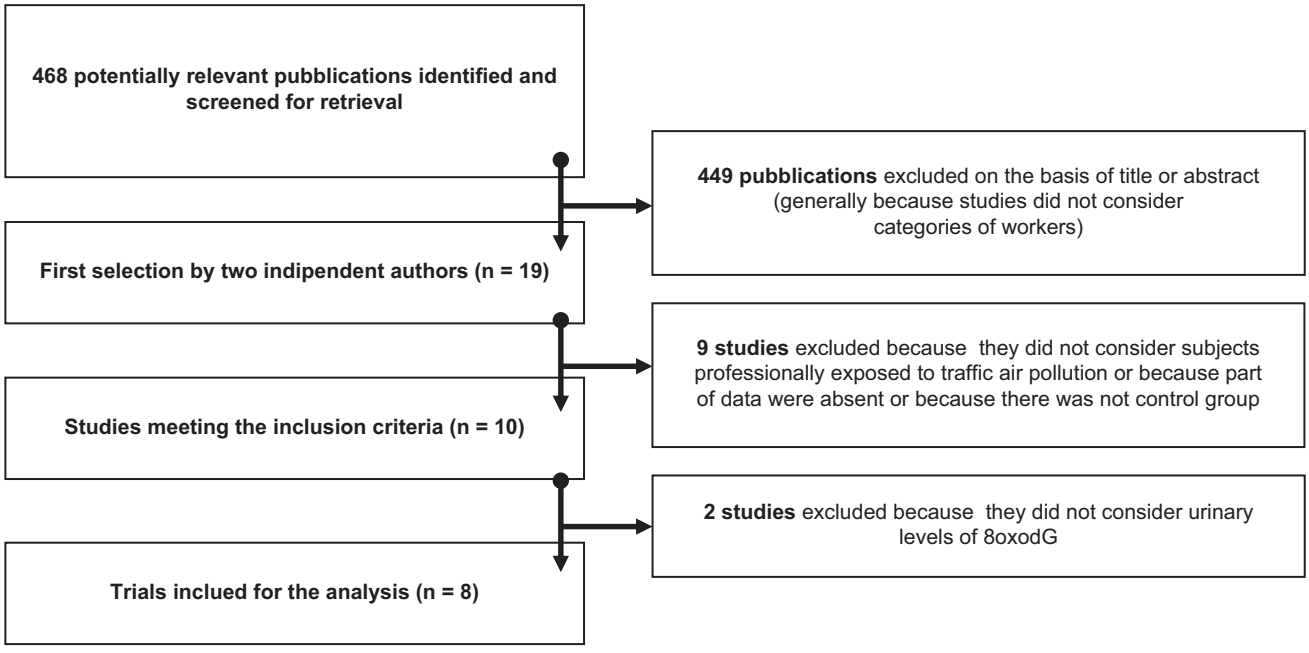

Figure 1. Flow diagram of the study selection process for meta-analysis.

Table 1. Studies included in our meta-analysis: characteristics and main results.

Authors	Group comparison: exposed vs not-exposed subjects	Method	Exposed smokers	Not-exposed smokers	Exposed non-smokers	Not-exposed non-smokers	Urinary 8oxod G levels in exposed subjects
			n, mean ± SD		*n*, mean ± SD		
Lai et al. 2005	Toll workers vs office workers	ELISA (μg g^{-1} creatinine)	*n=5* 10.2 ± 4.6	*n=8* 10.5 ± 8.2	*n=42* 13.6 ± 7.3	*n=16* 7.3 ± 4.8	↑ p <0.05
Lee et al. 2010	Station workers vs not-exposed workers	ELISA (μg g^{-1} creatinine)	*n=35* 14.05 ± 12.71	*n=2* 4.97 ± 3.49	*n=20* 14.05 ± 12.71	*n=30* 6.58 ± 4.39	↑ p <0.05
Rossner et al. 2007	Bus drivers vs healthy volunteers spending <90% of daily time indoors	ELISA (nmol $mmol^{-1}$ creatinine)			*n=50* 5.67 ± 2.50	*n=50* 3.82 ± 1.73	↑ p <0.01
Autrup et al. 1999	Bus drivers and postal workers vs bus drivers in rural area	Bus driversHPLC (nmol $mmol^{-1}$ creatinine)			*n=29* 1.74 ± 0.87	*n=20* 1.54 ± 0.96	↔
		Postal workers HPLC (nmol $mmol^{-1}$ creatinine)			*n=29* 2.25 ± 1.13	*n=20* 1.54 ± 0.96	
Harri et al. 2005	Garage workers vs Ns	HPLC (μmol mol^{-1} creatinine)	*n=26** 1.56 ± 1.38	*n=32** 1.50 ± 2.2			↔
Chuang et al. 2003	Taxi drivers vs general city population	ELISA (μg g^{-1} creatinine)	*n=55* 14.3 ± 5.3	*n=32* 13.3 ± 4.5	*n=40* 13.4 ± 4.7	*n=43* 11.5 ± 4.7	↑ p <0.05
Nilsson et al. 2004	Engine room personnel vs Ns	HPLC (nmol l^{-1} adjusted to density 1.022)	*n=9* 18.2 ± 5	*n=11* 10.8 ± 5	*n=6* 19.5 ± 10	*n=22* 17.6 ± 3	↔
Rossner et al. 2008	Bus drivers vs healthy volunteers spending <90% of daily time indoors	ELISA (nmol $mmol^{-1}$ creatinine)			*n=50* 7.16 ± 2.35	*n=50* 5.9 ± 2.4	↑ p = 0.01

HPLC, high-performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; Ns = Unspecified; * = mixed category (smokers and non smokers).

independently reviewed the search output of potentially relevant trials for inclusion and assessed the trial for inclusion. Studies not meeting the inclusion criteria were excluded. Disagreements were settled by discussion with a third co-author (A.S.).

Extracted data included the first author's name, year of publication, number of cases and number of controls, mean and SD, group comparison (exposed workers categories versus not-exposed subject categories) and the results of any single study included in our meta-analysis.

Assessment of quality characteristics used a number of criteria. They included: description of the degree of exposure to vehicle exhaust emissions for cases and controls (coded as adequate or inadequate); job description for exposed and not-exposed workers (coded as adequate or inadequate); dietary and smoking habits (coded as yes or no); evaluation of antioxidant status (coded as yes or no); and shift work (coded as yes or no) (Table 2). The studies were classified as: 'study of high quality', if all six components were included in each study; 'study of middle quality', if at least three components were included in each study and 'study of low quality', if less than three components were included in each study.

We did not create funnel plots because the limited number of trials included and the lack of studies with large sample sizes preclude a meaningful interpretation.

Data analysis

Relevant data of the studies available for formal meta-analytic evaluation were entered into the Comprehensive Meta-Analysis, software for evaluation of meta-analysis (Biostat, Englewood NJ, USA).

For each study, the core information comprised sample size, mean values and SDs of the respective outcome variables. When only the standard error of the mean (SEM) for a single variable was given, we computed the SD of the measures multiplying the SEM by the square

Table 2. Criteria used to assess the quality of studies included in our meta-analysis.

Items reported in each study		Laiet al. 2005	Leeet al. 2010	Rossner et al. 2007	Rossner et al. 2008	Autrup et al. 1999	Harri et al. 2005	Chuang |et al. 2003	Nilsson et al. 2004
Dietary habits	Yes		•					•	
	No	•		•	•	•	•		•
Smoking habits	Yes	•	•	•	•	•	•	•	•
	No								
Antioxidant status	Yes			•	•				
	No	•	•			•	•	•	•
Shift work	Yes	•			•		•		
	No		•	•		•		•	•
Degree of exposure to air pollution	Adequate			•	•	•	•	•	•
	Inadequate	•	•						
Job description	Adequate	•		•					
	Inadequate		•		•	•	•	•	•

root of the number of observations the mean value was based on. All outcome variables considered here were continuous. Group comparisons were performed between exposed and non-exposed workers as well as between smokers or non-smokers.

The outcome of the studies (urinary 8oxodG levels detected by HPLC or ELISA methods) was reported in different scales. To enable a joint comparison, in each individual study the standardized mean difference (SMD) between groups was calculated for each outcome. The SMD is the difference between the respective mean value divided by a common measure of dispersion which was computed from the SDs in the two groups (exposed and not-exposed workers). In our analysis a positive SMD indicates higher oxidatively damaged DNA in exposed workers.

A meta-analysis was performed using DerSimonian and Laird 'random effects models'. Under the random effects model, effect sizes are assumed not to be common to all studies, they vary under a normal distribution model (Higgins & Thompson 2002). Under this model, the weighted mean of the effect sizes (i.e. SMD) is computed, and a 95% confidence interval (CI) is determined.

We assessed heterogeneity with I^2, which describes the percentage of total variation across studies due to heterogeneity rather than chance. I^2 can be calculated as: $I^2 = 100\% - (Q-df)/Q$ (where Q = Cochrane's heterogeneity statistics, df = degrees of freedom). Negative values of I^2 equalled zero, so that I^2 ranged between 0% (i.e. no observed heterogeneity) and 100%. High values would show increasing heterogeneity (Higgins & Thompson 2002, Borenstein et al. 2009).

Results

Our meta-analysis included a total of 732 workers. They were divided in two groups: workers exposed to traffic air pollution (396 subjects) and workers not exposed to traffic air pollution (336 subjects). Each group was divided in two subgroups: smokers (215 subjects) and non-smokers (517 subjects). The working categories evaluated in our meta-analysis were: drivers (Rossner et al. 2007, 2008, Chuang et al. 2003, Autrup et al. 1999), toll-collectors (Lai et al. 2005), postmen (Autrup et al. 1999), engine drivers (Nilsson et al. 2004), and garage and filling station workers (Harri et al. 2005, Lee et al. 2010). In five studies the urinary 8oxodG levels were detected by ELISA, whereas in three studies an HPLC assay was utilized. Among the included studies in our meta-analysis, five reported increases in urinary 8oxodG levels in exposed workers whereas in three studies no effect was found (Table 1). Among the eight studies included in our meta-analysis, 75% were of middle quality (Table 2).

Our main aim was to evaluate if occupational exposure to vehicle exhaust emissions could modulate urinary 8oxodG concentration. In the forest plot in Figure 2, we show the effect of traffic air pollution on urinary 8oxodG concentration in non-smokers and smokers, comparing the exposed and not-exposed groups. The resulting SMD (random effects model) of the first category (non-smoker exposed workers) was 0.659 (95% CI 0.460–0.858) in units of standard deviations and the *p*-value from the corresponding test against an overall null effect of traffic air pollution was less than 0.001. In the smokers category, the resulting SMD was 0.279 (95% CI 0.05–0.508) and the *p*-value was 0.046. The degree of inconsistency (I^2) among the studies was 0% and 25%, respectively, for the non-smoker and smoker groups.

In Figure 3, urinary 8oxodG concentration in the same subjects was evaluated by comparing HPLC and ELISAs. For the HPLC assay, the resulting SMD was 0.399 (95% CI 0.094–0.704) and the *p*-value was 0.014, whereas the resulting SMD observed for ELISA assay was 0.571 (95% CI 0.368–0.774) in units of standard deviations and the

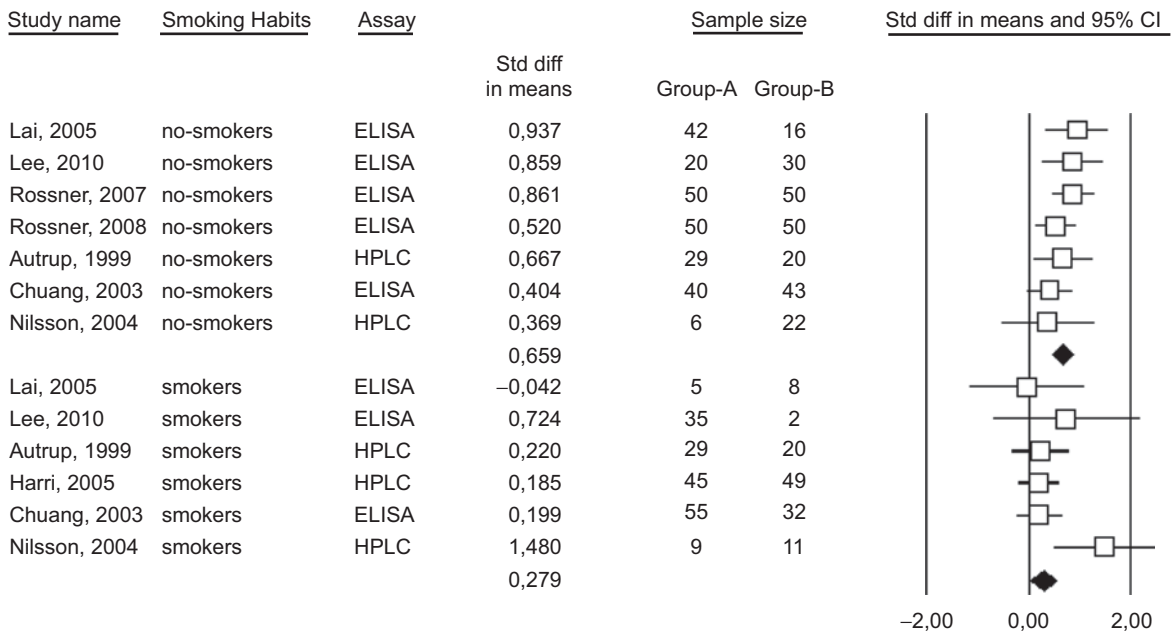

Figure 2. The effect of exposure to traffic air pollution on urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) levels in exposed workers, in relation to smoking habits. A positive standardized mean difference (SMD) indicates higher oxidatively damaged DNA in exposed workers. Group A: exposed subjects; group B: not-exposed subjects.

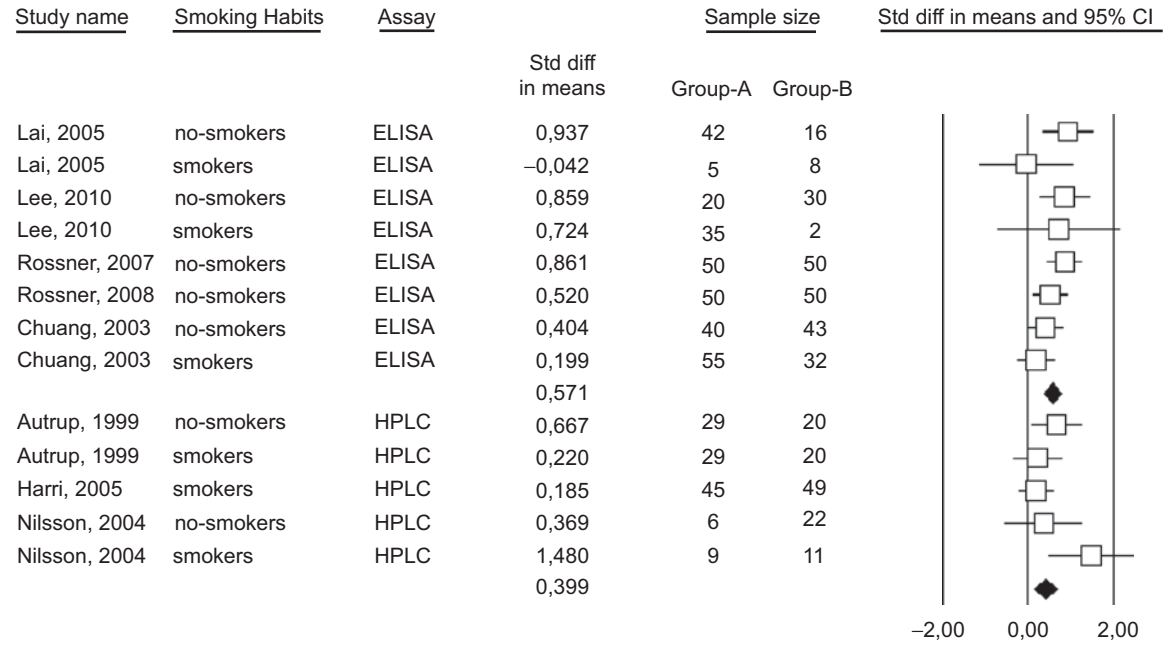

Figure 3. The effect of exposure to traffic air pollution on urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) levels in exposed workers, in relation to the method used in each study. A positive standardized mean difference (SMD) indicates higher oxidatively damaged DNA in exposed workers. Group A: exposed subjects; group B: not-exposed subjects.

p-value from the corresponding test against an overall null effect of traffic air pollution was below 0.001. The I^2 was 41% and 22%, respectively, for the HPLC and ELISAs.

The comparison in the assay (HPLC vs ELISA) was stratified in relation to smoking habits (Figures 4 and 5). In the non-smoking category, the resulting SMD was 0.580 (95% CI 0.08–1.071; p <0.021 and I^2 0%) and 0.673 (95% CI 0.222–0.895; p <0.001 and I^2 9%) for HPLC and ELISA assays, respectively. On the other hand, in the smoking category we observed that SMD was 0.325 (95% CI 0.011–0.639; p <0.042 and I^2 65%) and 0.209 (95%

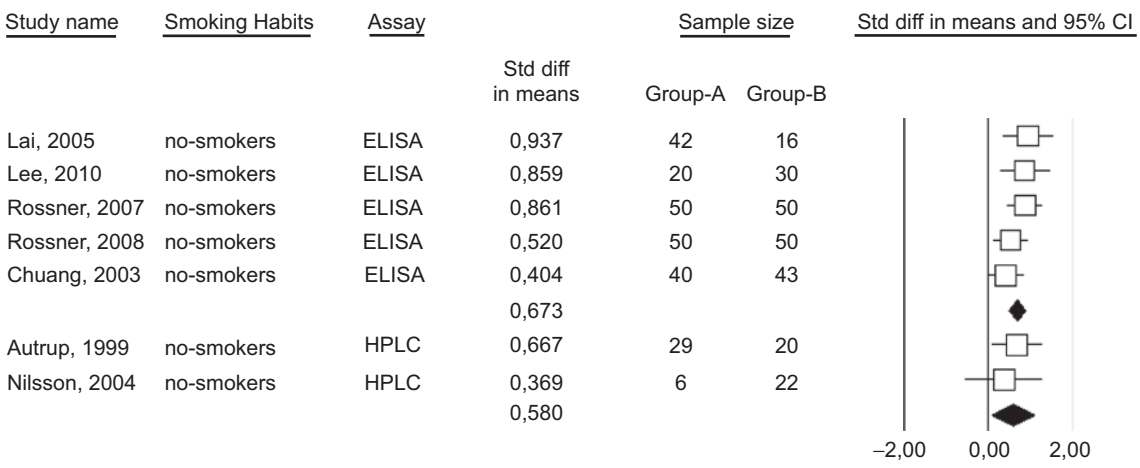

Figure 4. The effect of exposure to traffic air pollution on urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) levels in exposed workers, in relation to the method used in non-smokers. A positive standardized mean difference (SMD) indicates higher oxidatively damaged DNA in exposed workers. Group A: exposed subjects; group B: not-exposed subjects.

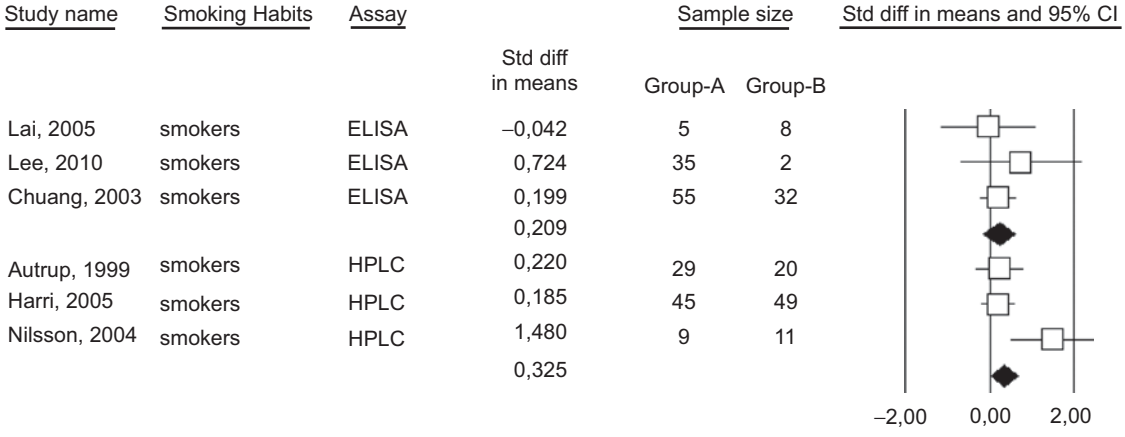

Figure 5. The effect of exposure to traffic air pollution on urinary 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) levels in exposed workers, in relation to the method used in smokers. A positive standardized mean difference (SMD) indicates higher oxidatively damaged DNA in exposed workers. Group A: exposed subjects; group B: not-exposed subjects.

CI -0.183 to 0.600; p <0.296 and I^2 0%) in the HPLC and ELISA assays, respectively.

Discussion

Studies conducted over the past 15 years, have reported that chronic exposure to air pollutants is associated with increased morbidity and mortality (Jerrett et al. 2009). A major component that contributes to air pollution is exhaust from vehicles. Although several explanations have been offered as to the mechanism by which air pollution produces its harmful effects, the exact mechanism is still not clear. Recent studies have suggested that traffic-related air pollution particles can cause an imbalance in the endogenous redox status towards an increase of pro-oxidant species in humans. This condition is to be regarded as one of the mechanisms through which air pollution promotes adverse effects on human health (Yang & Omaye 2009).

Several studies have identified subjects occupationally exposed to vehicle exhaust emissions, as a category particularly vulnerable to oxidative insults (Suresh et al. 2000, Singh et al. 2008). In the context of preventive medicine, studies aimed at identifying exposure biomarkers capable of predicting an increased risk of diseases, including cancer, are consistent. Among the major biomarkers of oxidatively generated DNA damage detected in subjects exposed to air pollutants, urinary 8oxodG has received particular interest (Møller et al. 2008). Recently, measuring urinary 8oxodG has become more popular as a means of assessing oxidative stress in humans because urinary 8oxodG probably reflects increased pro-oxidant species

and a decrease in body's antioxidant defence system. Although HPLC and ELISAs are more reliable methods to measure urinary 8oxodG levels, the significant discrepancy between HPLC and ELISA results may create obstacles to their use in many molecular epidemiological studies (Cooke et al. 2009b).

In some literary studies, diet was suggested to contribute to urinary levels of thymine glycol and 8-oxo-7,8-dihydro-guanine (8oxoGua), but more recent data suggest that urinary 8oxodGua levels are not affected by diet, removing this issue from possible confounders (Evans et al. 2010, Cooke et al. 2000, Gackowski et al. 2001). Moreover, the results about the impact of body mass index on urinary 8oxodG concentration are contrasting (Mizoue et al. 2006, 2007, Pourcelot et al. 1999, Davanipour et al. 2009). On the other hand, a large body of evidence shows increased urinary 8oxodG levels in subjects with chronic diseases (e.g. metabolic syndrome) (Allen et al. 2009).

The aspects described above, were considered in order to develop the inclusion/exclusion criteria in our meta-analysis.

In relation to the impact of traffic air pollution on oxidatively generated DNA damage, in the literature there is no significant evidence of an increase in urine 8oxodG levels in exposed subjects (Singh et al. 2008, Rossner et al. 2007, Chuang et al. 2003). This is in accordance with our systematic review of all published case-controlled studies where urinary 8oxodG levels in exposed workers were measured. In five studies an increase in urinary 8oxodG levels was observed and in three studies no effect was found in exposed workers. However, a low statistical power can be the reason for the failure of some studies in demonstrating a traffic air pollution effect on urinary 8oxodG levels; and a meta-analysis with rigorous inclusion criteria can help by bringing together the available studies.

Considering that cigarette smoke can be an important confounding factor in occupational studies (Harri et al. 2005, Mizoue et al. 2007), assessing the effect of air pollutants by measuring urinary 8oxodG levels, we analysed the results of each study dividing all subjects in relation to their smoking habits.

The first result of our meta-analysis showed an increase in urinary 8oxodG levels both in smokers and in non-smokers. However, in the smoker category, we observed an increase in 8oxodG levels within statistically significant limits ($p = 0.046$). In accordance with other studies, cigarette smoking could mask the effect of air pollutants on urinary 8oxodG because it also causes a significant increase in pro-oxidant species in smokers (Chuang et al. 2003, Hoffmann et al. 2005). These results can be better evaluated by analysing Figures 4 and 5. In particular, analysing the impact of air pollution in relation to the method used in each study, an interesting difference was observed between ELISA and HPLC assays in the smoker categories (Figure 5). A significant increase in oxidatively generated DNA damage was observed only in studies that used an HPLC assay to detect the urinary 8oxodG concentration in exposed subjects who smoked, whereas no difference between exposed and not-exposed smokers was observed when the ELISA was used. On the other hand, in Figure 4 we showed increased levels of 8oxodG in the urine of exposed workers who did not smoke using ELISA as well as a HPLC assay, even if the latter method was used only in two studies.

Our results identified urinary 8oxodG as a biomarker of use in evaluating the pro-oxidant effect of vehicle exhaust emissions on DNA in healthy workers. Moreover, our data point out: (1) the need to calculate the cigarette smoking habit in the studies evaluating the impact of traffic air pollution on urinary 8oxodG levels; and (2) the possibility of applying ELISA assay to studies comparing relative urinary 8oxodG value among several groups, when the same studies are not used to calculate the exact concentration of 8oxodG in urine.

In conclusion, with our homogeneous population of exposed subjects (workers free from disease and normal weight), stratified in relation to cigarette smoking habit, we have provided for the first time, a useful 'benchmark' for future studies looking at the impact of traffic air pollution on urinary 8oxodG levels in subjects occupationally exposed.

Declaration of interest

The authors report no conflicts of interest.

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