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Serum Oxidant and Antioxidant Levels in Diesel Exposed Toll Collectors

Peri ARBAK[1], Ozlem YAVUZ[2], Neslihan BUKAN[3], Oner BALBAY[1], Füsun ULGER[4] and Ali Nihat ANNAKKAYA[1]

[1]Department of Chest Diseases, [2]Department of Biochemistry, Abant Izzet Baysal University, Duzce School of Medicine, [3]Department of Biochemistry, Gazi University, School of Medicine and [4]Department of Chest Diseases, Ankara University, School of Medicine, Turkey

Abstract: Serum Oxidant and Antioxidant Levels in Diesel Exposed Toll Collectors: Peri ARBAK, *et al.* Department of Chest Diseases, Abant Izzet Baysal University, Duzce School of Medicine, Turkey—It has been suggested that exposure to diesel exhaust may lead to adverse effects due to the generation of oxidants. To evaluate the end products of oxidative stress in DE exposure, toll collectors who are considered a high risk group in regard to occupational toxins were compared to controls who had office-based occupations in the same company in this cross sectional study. A total of 38 toll collectors constituted the study group. All subjects were male. The toll collectors and 29 controls were similar regarding age, smoking status and duration of work. All subjects underwent a clinical examination and an interviewer-administrated questionnaire regarding respiratory symptoms, past medical and occupational history, and pulmonary function tests were performed in all subjects. Serum malondialdehyde (MDA), nitrite+nitrate and vitamin E levels were measured. Toll collectors showed higher serum MDA (5.76 ± 2.15 μmol/L vs. 3.07 ± 0.76 μmol/L, p=0.0001) and nitrite+nitrate levels (96.50 ± 45.54 μmol/L vs. 19.32 ± 11.77 μmol/L, p=0.0001) than controls. Vitamin E levels were similar in toll collectors and controls (10.57 ± 3.44 mg/L and 9.72 ± 2.44 mg/L, respectively, p=0.267). There was no difference between groups in terms of the findings of clinical examinations and respiratory symptoms. In pulmonary function parameters, only peak expiratory flow (PEF) in toll collectors was significantly lower than that of controls (88.9% predicted and 104.2% predicted, respectively, p=0.012). In conclusion, we suggest that serum MDA and nitrite+nitrate levels may be used as biological markers of oxidative stress related to DE exposure, but prospective controlled clinical studies are necessary to clarify the possible association between concentrations of MDA and nitrite+nitrate and pulmonary diseases related to DE exposure.
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Key words: Occupation, MDA, Nitrite+nitrate, Vitamin E, Toll collectors, Diesel exhaust

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Correspondence to: P. Arbak, Department of Chest Diseases, Abant Izzet Baysal University, Duzce School of Medicine, Konuralp, Duzce, 81620, Turkey (e-mail: periarbak@hotmail.com)

Diesel exhaust (DE) has been accused of various health outcomes including exacerbation of asthma, chronic bronchitis, respiratory tract infections and ischaemic heart diseases[1)]. It has been suggested that the adverse health effects of DE are associated with the generation of various oxidants.

In an *in vivo* toxicity study, intratracheally instilled DEPs (Diesel exhaust particles) generated reactive oxygen species (ROS) in the lungs of exposed mice[1)]. Moreover, studies of lung microsomes have shown that redox cycling quinones were involved in superoxide generation by DEPs[2)]. The mitochondrial pathway was also involved in the generation of superoxide by organic DEP extracts[2)]. Once produced, ROS can degrade proteins, promote DNA- strand breakage and compromise the integrity of polyunsaturated fatty acids[3)]. Clinical consequences of ROS interactions are thought to be associated with different diseases including those of the cardiovascular, neurological and pulmonary systems[4–6)].

In the pulmonary system, ROS causes airway and parenchymal injury by the induction of abnormal inflammatory response and impairment of the barrier function of endothelial and epithelial cells[4)].

The levels and activity of ROS are controlled by enzymatic defence mechanisms such as superoxide dismutase, glutathione peroxidase, catalase and non-enzymatic defence mechanisms such as ascorbic acid,

vitamin E and glutathione[7].

Therefore, it can be postulated that a decrease in the level of either enzymatic or non-enzymatic defence markers of antioxidants may reflect an increase in oxidative stress.

DEP_s account for a highly significant percentage of the motor vehicle generated air pollutants.

Toll collectors who work in motorways constitute a population with considerable occupational exposure to DE. The principle objective of the current study was to investigate the levels of several serum markers of oxidative stress and vitamin E in this occupational group.

Materials and Methods

Groups

The design of the current study was approved by the Ethical Committee of our institution. A written informed consent was signed by all participants.

The study was conducted between September and December 2002 in a rural area. The exposed group consisted of 38 (mean age: 33.1 ± 6.1) toll collectors working at the stations of motorway junctions in this rural area. All these workers were male, and they were found eligible out of the total number of 50 toll collectors. Eight toll collectors refused to be in this study. The remaining 4 toll collectors who have systemic diseases such as hypertension, diabetes mellitus, chronic obstructive pulmonary disease and asthma were excluded. The controls were selected out of 34 office-workers in the same company. Among the controls 5 were excluded due to refusal. The mean age of the controls was 34.6. Two groups were also similar with respect to smoking habit, and antecedent medical and occupational history.

All men in the exposed and control groups underwent full physical examination by the same physician. The clinical and occupational histories were obtained by one physician using an open questionnaire modified from Ferris[8]. It included the following items: demographical details including age and gender, type and the duration of work exposure, work status at the time of assessment, the nature and frequency of respiratory symptoms (chest tightness, wheezing, dyspnea, cough, sputum), smoking habit and detailed history of previous and current respiratory diseases. Peripheral blood samples were obtained for the measurement of plasma malondialdehyde, nitrite+nitrate and Vitamin E levels. Blood samples were collected from both groups under the same circumstances including the time of the procedure and physical conditions to exclude a possible diurnal variation in these serum markers. All samples were immediately stored in a refrigerated box and kept at ~4°C until their arrival at university hospital within one hour. An aliquot of blood was centrifuged at 1,700 × g (Relative Centrifugal Force) for 15 min at room temperature. Serum samples were partitioned and frozen at –40°C until analysis.

The week after the first medical evaluation in the workplace all the subjects in the exposed and control groups were invited to the radiology department to obtain a chest radiograph.

Spirometric measurements

Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), maximum mid-expiratory flow (MEF_{25-75}) and peak expiratory flow (PEF) were measured in all subjects with a spirometer (Vitalograph Alpha). Three technically acceptable measurements were made for each subject and all volumes were corrected for body temperature and pressure saturation (BTPS). Results were expressed as percentages of the predicted values[9].

Measurement of MDA

The quantity of thiobarbituric acid reactive substances (TBARS) in the serum samples as an index of malondialdehyde (MDA) production and hence lipid peroxidation, was determined by the method described by Yoshioka *et al*.[10]. After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in n-butanol and was measured spectrophotometrically at 532 nm. 1,1,3,3-tetraethoxypropane was used as the standard. MDA levels were expressed as μmol/L.

The detection limit of the method was 0.5–1 μmol/*l* in the sample and coefficient variation (CV) of the method was 4%. The TBARS values were calculated with the molar extinction coefficient of the MDA-TBA complex of 1.5 × 106 cm–1 m–1 as previously described[11].

Measurement of Nitrite

Nitrite concentrations were measured by using the Griess reaction[12]. Briefly, serum samples were diluted in distilled water and deproteinized by adding zinc sulfate. After centrifugation, the supernatant was applied to a microtiter plate well, followed by Griess reagent (1 g/L sulfanilamide, 25 g/L phosphoric acid and 0.1 g/L N-1-naphthylethylenediamine). The absorbance was measured at a wavelength of 540 nm. The results were expressed as μmol/L.

Measurement of Nitrate

Nitrate concentrations were measured as nitrite after enzymatic conversion by nitrate reductase as described by Schmidt *et al*[13]. Briefly, plasma was diluted with distilled water. NADPH, FAD and nitrate reductase were added. Samples were subsequently incubated at 37°C and then mixed with lactate dehydrogenase and sodium pyruvate. Samples were further incubated at 37°C to oxidize NADPH, deproteinized and assayed with Griess reagent as described above. Values obtained by this procedure represent the sum of nitrite and nitrate. Nitrate concentrations were obtained by subtracting nitrite

concentrations from the total nitrate + nitrite concentrations. In a previous study, normal values for nitrate + nitrite were determined in serum samples (median 22.2 μmol/L; range 8.4–48.7 μmol/L)[14].

Measurement of vitamin E

The vitamin E concentration in serum was analysed as previously described[15]. Briefly, after deproteinization with ethanol, serum was extracted with hexane. Fluorescence was then measured in the supernatant in a Shimadzu RF-5301 PC spectrofluorophotometer, the excitation wavelength was 295 nm and emission wavelength was 340 nm. The values were expressed as mg/L. Normal values for vitamin E were 5–20 mg/L[15].

Statistical analysis

An SPSS-10.0 program was used for statistical analysis. Group means were compared by Student's t-test or Mann-Whitney U test and frequencies were compared by Chi-squared or Fisher's exact test. Results were considered statistically significant if $p<0.05$.

The comparison of mean levels of MDA, nitrite+nitrate, vitamin E in different subgroups (smokers vs nonsmokers, symptomatics vs nonsymptomatics) of toll collectors and controls was performed with two-way independent ANOVA. Thus, interaction effects between smoking and group, each symptom (cough, sputum, dyspnea, wheezing, chest tightness) and group on the mean levels of MDA, nitrite+nitrate and vitamin E were evaluated. A p value less than 0.05 was considered statistically significant.

Results

The characteristics of toll collectors and controls are given in Table 1. There were no significant differences among these groups with respect to age, smoking habit, the duration of work or the presence of respiratory symptoms ($p>0.05$).

In pulmonary function parameters, only peak expiratory flow (PEF) in toll collectors was significantly lower than that of controls (88.9% predicted and 104.2% predicted, respectively, $p=0.012$).

No abnormal findings were observed in chest radiographs of toll collectors or controls. Chest graphs were evaluated by three observers, one of whom is from the radiology department and two from the chest department.

Three toll collectors (8%) and three controls (10%), all of whom were smokers, presented with rhonchi on clinical examination. No significant difference was observed between groups according to the presence of rhonchi ($p=0.526$). Further investigations did not reveal chronic obstructive lung diseases or asthma.

The plasma MDA and nitrite + nitrate levels are shown

Table 1. Characteristics of toll collectors and controls who participated in the study

	Toll collectors n=38	Controls n=29	p
Age (yr)	33.1 ± 6.1	34.6 ± 5.9	0.318
Duration of work (yr)	9.3 ± 4.4	10.2 ± 5.3	0.441
Smokers (%)	71.1	79.3	
Exsmokers (%)	7.9	13.8	0.234
Nonsmokers (%)	21.0	6.9	
Mean pack-yr of smokers	15.2 ± 8.6	11.5 ± 7.8	0.126
FVC (% pred)	105.3 ± 8.6	101.0 ± 11.8	0.278
FEV_1 (%pred)	109.9 ± 18.2	106.9 ± 13.8	0.467
MEF_{25-75} (%pred)	106.9 ± 26.6	109.8 ± 25.8	0.657
PEF (% pred)	88.9 ± 26.5	104.2 ± 20.4	0.012*
Respiratory symptoms			
Cough (%)	42.1	37.9	0.464
Sputum (%)	50.0	34.5	0.154
Wheezing (%)	36.8	20.7	0.122
Chest tightness (%)	36.8	34.5	0.524
Dyspnea (%)	34.2	20.7	0.173

Results are expressed as the mean± SD or as a percentage . FEV_1: forced expiratory volume in one second, FVC: forced vital capacity, MEF_{25-75}: maximum mid expiratory flow, PEF: peak expiratory flow, % pred: percentage of predictive value, *$p<0.05$, group means were compared by Student's t-test and frequencies were compared by Chi-squared test

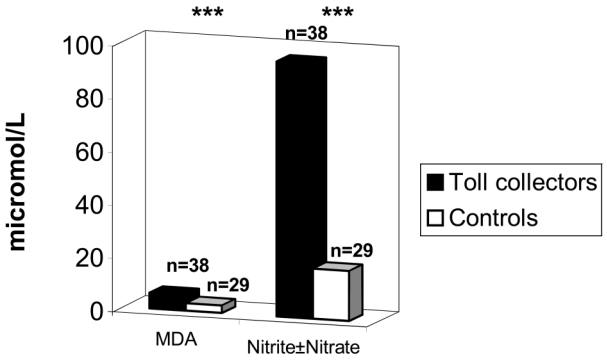

Fig. 1. A comparison of serum MDA and nitrite+nitrate levels in study (toll-collectors) and control groups. The mean serum MDA and nitrite+ nitrate levels were significantly higher in toll collectors than in controls (***p<0.001).

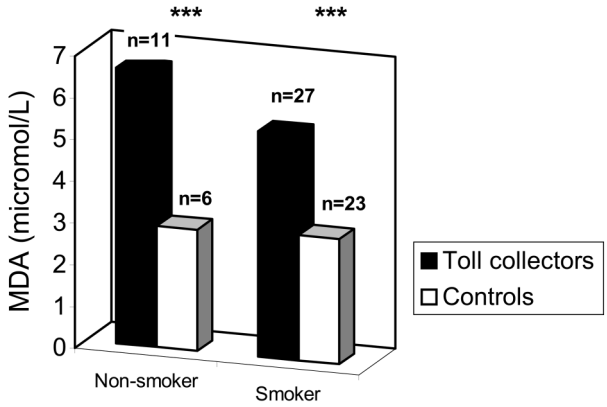

Fig. 2. MDA levels of toll collectors were significantly higher than those of controls both in non-smokers and smokers (***p<0.001). MDA levels of smokers were not significantly higher than those of non-smokers either in toll collectors or controls (p>0.05). There was no significant interactive effect between smoking status and group, on the mean MDA level (p>0.05).

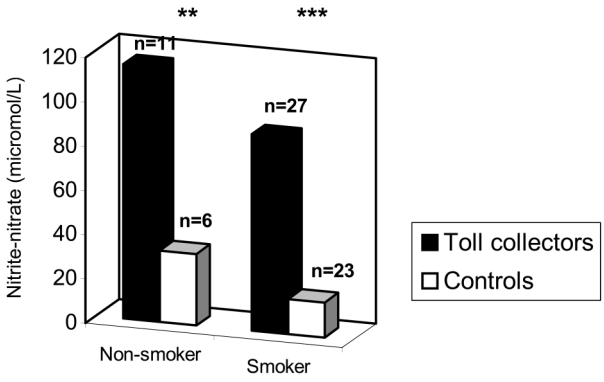

Fig. 3. Nitrite+nitrate levels of toll collectors were significantly higher than those of controls both in non-smokers and smokers (**p<0.01 and ***p<0.001, respectively). Nitrite+nitrate levels of smokers were not significantly higher than those of non-smokers either in toll collectors or controls (p>0.05). No significant interactive effect between smoking status and group, on the mean nitrite+nitrate level was observed (p>0.05).

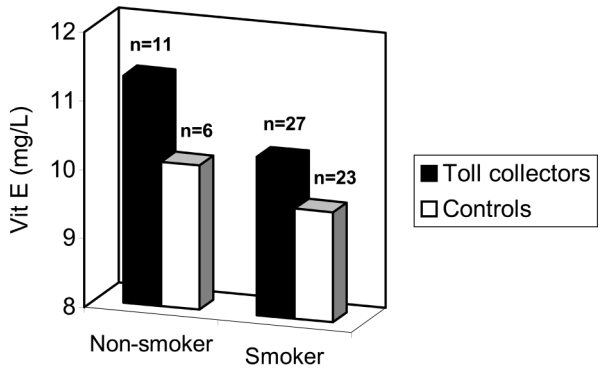

Fig. 4. Mean vitamin E levels of both smokers and nonsmokers among toll collectors and controls. No significant interaction effect between smoking status and group on the mean vitamin E level was observed (p>0.05).

in Fig. 1. The mean serum MDA and nitrite+ nitrate levels were significantly higher in the exposed group (5.76 μmol/L and 96.50 μmol/L) than in the controls (3.07 μmol/L and 19.32 μmol/L) (p=0.0001 and p=0.0001, respectively).

With respect to Vitamin E levels, no statistically significant difference was observed between toll collectors and controls (10.57 mg/L and 9.72 mg/L, respectively) (p=0.267).

The mean MDA levels of smoker and nonsmoker subgroups of toll collectors and controls are shown in Fig. 2. Both smoker (mean MDA=5.4 ± 2.1 μmol/L) and nonsmoker (mean MDA=6.6 ± 2.0 μmol/L) subgroups of toll collectors had higher mean MDA levels than those of controls (smokers mean MDA=3.0 ± 6.6 μmol/L and nonsmokers mean MDA=2.9 ± 1.1 μmol/L). There was no significant interactive effect between smoking status and group, on the mean MDA level (p>0.05).

As shown in Fig. 3, the mean nitrite+nitrate levels in smokers (88.9 ± 45.2 μmol/L) and nonsmokers (115.0 ± 42.6 μmol/L) of toll collectors were higher than those of controls (15.9 ± 8.5 μmol/L and 32.3 ± 15.9 μmol/L, respectively). No significant interactive effect between

Table 2. The participants with symptoms versus participants without symptoms of toll collectors and controls regarding mean MDA, nitrite+nitrate and vitamin E levels

Symptoms	MDA (μmol/L)		Nitrite+Nitrate (μmol/L)		Vit E (mg/L)	
	Toll collectors	Controls	Toll collectors	Controls	Toll collectors	Controls
Cough						
positive	5.5 ± 1.9	2.9 ± 0.7	93.0 ± 48.0	21.1 ± 8.8	11.2 ± 3.2	9.4 ± 2.2
negative	5.9 ± 2.3	3.1 ± 0.7	99.0 ± 44.6	18.2 ± 13.3	10.1 ± 3.6	9.9 ± 2.6
Sputum						
positive	5.6 ± 1.8	2.9 ± 0.5	88.3 ± 35.8	18.9 ± 12.2	11.3 ± 4.2	10.2 ± 2.3
negative	5.9 ± 2.4	3.1 ± 0.8	104.6 ± 53.3	19.5 ± 11.9	9.8 ± 2.3	9.5 ± 2.5
Wheezing						
positive	6.4 ± 2.9	3.1 ± 0.8	105.4 ± 44.3	14.8 ± 8.4	11.8 ± 3.7	11.3 ± 2.6
negative	5.3 ± 1.4	3.0 ± 0.7	91.3 ± 46.4	20.5 ± 12.9	9.8 ± 3.1	9.3 ± 2.7
Dyspnea						
positive	5.4 ± 2.1	2.8 ± 0.3	88.4 ± 46.2	17.1 ± 15.9	11.9 ± 3.8	10.0 ± 3.2
negative	5.9 ± 2.2	3.1 ± 0.8	100.7 ± 45.6	19.8 ± 10.8	9.8 ± 3.0	9.6 ± 2.2
Chest Tightness						
positive	6.1 ± 3.0	3.1 ± 0.7	110.5 ± 45.7	15.5 ± 8.5	11.9 ± 3.8	10.0 ± 3.2
negative	5.5 ± 1.4	3.0 ± 0.8	88.3 ± 44.3	21.3 ± 12.9	9.7 ± 2.9	9.5 ± 2.1

No interactive effects between each symptom (cough, sputum, dyspnea, wheezing, chest tightness) and group, on the mean levels of MDA, nitrite+nitrate and vitamin E were observed ($p>0.05$).

smoking status and group, on the mean nitrite+nitrate level was observed ($p>0.05$).

As shown in Fig. 4 comparable vitamin E levels were detected in smokers (10.2 ± 3.2 mg/L) and nonsmokers (11.3 ± 4.0 mg/L) among toll collectors and controls (9.6 ± 2.5 mg/L and 10.1 ± 2.1 mg/L for smokers and nonsmokers, respectively). No significant interactive effect between smoking status and group on the mean vitamin E level was observed ($p>0.05$).

The participants with symptoms versus participants without symptoms among toll collectors and controls regarding mean MDA, nitrite+nitrate, vitamin E levels are seen in Table 2. The mean MDA, nitrite + nitrate and vitamin E levels in both the participants with symptoms and participants without symptoms among toll collectors were higher than those of controls. No interactive effects between each symptom (cough, sputum, dyspnea, wheezing, chest tightness) and group, on the mean levels of MDA, nitrite + nitrate and vitamin E were observed ($p>0.05$).

There were no subjects with a PEF value below 65% of predicted in controls, whereas nine out of 38 toll collectors had a PEF value less than 65% of that predicted ($p=0.004$).

Discussion

Several animal studies showed a clear linkage between DEP exposure and excessive production of ROS[1, 16], and DEP ambient levels can be relatively higher in some locations; selected populations such as toll collectors are thought to be higher risk groups for DEP exposure. In this study, too see whether there was any relationship between DEP exposure and byproducts of peroxidation in a specific high-risk group, toll collectors were investigated.

We measured the serum levels of several markers for oxidative injury and vitamin E to assess the possible detrimental role of this particular occupation. Consequently, we demonstrated a significantly increased serum MDA and nitrite+nitrate levels in toll collectors when compared to controls. To the best of our knowledge, this is the first study that investigated the serum MDA levels in an occupational group exposed to DE.

The measurement of malondialdehyde (MDA) levels is the most widely used method when assessing lipid peroxidation[6]. Malondialdehyde is an endproduct of the oxidation of polyunsaturated fatty acids and is thought to be a result of oxidative stress[6, 7, 17]. Increased plasma MDA levels are detected in neurodegenerative diseases, smokers, HIV infection, inflammatory bowel disease and Syndrome X[5, 6, 18]. Moreover, it has been demonstrated that the metabolites (MDA and acetaldehyde) derived from ethanol and cigarette smoke could form adducts that stimulate airway epithelial cell protein kinase C (PKC) mediated release of various cytokines such as IL-8. Smoking related problems are thought to be due to the production of MDA[19]. In the present study, there were no significant differences among groups with respect to smoking habit and socioeconomical status. Moreover, when we compared the mean MDA levels of smokers

and nonsmokers in toll collectors and controls, both smoker and nonsmoker toll collectors had higher mean MDA levels than those of controls. No interactive effect was observed between smoking status and group, on MDA levels. Although smoking has been shown to have an increased effect on MDA levels in several studies[20, 21], in the current study it seemed unlikely that the increased serum MDA levels in toll collectors were related to smoking. Surprisingly, insignificantly higher MDA levels in nonsmoker toll collectors than in smoker toll collectors may be directly related to DEP exposure, but a large study group is needed to draw a conclusion. Furthermore, no interactive effect between participants with or without symptoms, on MDA levels was observed. It is also known that DEP and oxidative stress lead to the activation of the NF-κB and mitogen-activated protein kinase cascades in macrophage and epithelial cells and activate cytokines that contribute to bronchial hyperresponsiveness in humans[2]. Although a striking increase in plasma MDA levels was observed in toll collectors, pulmonary function tests of toll collectors and controls did not show any difference except for PEF. According to its dependence both on the effort of the subject and performance of technician, PEF was not accepted as a reliable parameter when evaluating the deleterious effects of DEP on airways. $MEF_{25-75\%}$ (maximal mid expiratory flow), which is less effort-dependent than the FEV_1 and the PEF and is a more sensitive indicator of early airway obstruction, is similar in two groups. In a study by Salvi *et al*; a pronounced inflammatory response was detected in human airways, whereas lung function parameters were found unaffected after exposure to DE. The investigators suggested that lung function measurements alone could not be used to exclude adverse air-pollution-associated airway responses[22]. Although no changes in pulmonary function tests were observed in our study, inflammatory responses might have been detected if we had performed bronchoalveolar fluid analysis.

The nitric oxide radical (NO) is increasingly recognized as an important intra and intercelluler messenger[23]. The role of NO has been implicated in a variety of biological processes such as neurotransmission, tumor cell killing, immunity and inflamatory processes[24]. Both cytoprotective and cytotoxic effects of NO have been reported[25]. When NO is formed by inducible NO synthase (iNOS), it is involved in inflammatory responses and pathogen killing[24]. NO is an extremely unstable molecule and is rapidly converted *in vivo* and *in vitro* to nitrate (NO3-) and nitrite (NO2-). Therefore, serum nitrite and nitrate have been used as indices of NO generation[14]. Reactive oxygen species are mainly derived from superoxide, whereas reactive nitrogen species formation mostly starts with the synthesis of nitric oxide. Superoxide is released during the respiratory burst of granulocytes and macrophages by NADPH oxidase activity[26]. Interaction of superoxide and NO leads to the production of more reactive oxidants such as peroxynitrite. DE contains a great amount of gases such as carbon monoxide (CO), nitric oxides (NO, NO2), sulphur dioxide (SO2), hydrocarbons, formaldehyde, transition metals and carbon particles[1, 27]. Besides causing irritation of the upper respiratory tract, the gaseous component of DE is able to penetrate the epithelium and vascular walls[1]. Our study group had higher MDA levels and nitrite+nitrate levels than those of controls. Although serum nitrite and nitrate levels can also reflect the dietary intake rather than NO metabolism *in vivo*[28] it is still unknown how a number of factors, apart from airway inflammation, can influence NO levels. Similarly, cigarette smoke is known to contain NO, and therefore it is reasonable to assume that an increase in NO are transiently induced by cigarette smoking. This hypothesis has been supported by several animal studies. But in another study, smoking a single cigarette temporarily decreased nitrate, nitrite, and serum antioxidant concentrations in plasma[29].

As shown in a previous study[30] nitrate in the diet seems to substantially influence the levels of exhaled NO (ENO). It is important either to restrict or register the intake of nitrate-rich food prior to measuring ENO, but in the present study, toll collectors and controls were in similar feeding facilities supplied by the same company, environmental and socioeconomical conditions. We suggested that increased plasma nitrite+nitrate levels in toll collectors might be dependent on penetration of the gaseous components of DE. The lack of facilities for measuring the level of the gaseous components of DE and DEP in manual tollbooths was the major limitation of our study.

According to the results of the current study, an increased MDA level due to lipid peroxidation was observed in toll collectors, whereas vitamin E levels did not differ significantly in the two groups. Lipid peroxidation is a complicated radical chain reaction leading to the formation of various products including MDA[31]. Vitamin E, a lipid-soluble vitamin, is believed to be the first line of defense against cell membrane damage due to peroxidation. It scavenges free radicals, terminating chain reactions and confining damage to a limited area of the membrane[32]. In different clinical conditions including smoking, human immunodeficiency virus (HIV) infection and inflammatory bowel disease, it has been shown that lipid peroxidation parameters, such as plasma lipid peroxides (LPO) and MDA are increased, whereas the levels of antioxidant micronutrients such as selenium, vitamins E and C, beta-carotene and carotenoids are reduced[18]. Although several studies have shown that antioxidants including vitamin E increase with oxidative stress and exercise training, this increase in

antioxidant defenses might not be physiologically proportionate to the needs created by the increase in oxidant events[33]. In a previous study, it was determined that the plasma vitamin E levels in patients with coronary artery disease were not significantly different from those in controls and these patients also had higher MDA levels[34]. In addition, the correlation between oxidative stress and aging was investigated and an age related increase in lipid peroxidation expressed as MDA was found, whereas a decline in antioxidant status except glutathione peroxidase (GPx) was not determined[35]. In the present study, the plasma concentrations of vitamin E in toll collectors and controls were in the normal ranges. The unchanged vitamin E levels in toll collectors might be due to an adaptation effect on the increased oxidant levels during DE exposure or due to a protection against oxidative stress by scavengers other than vitamin E. In a study by Robertson *et al*; highly trained runners had the highest erythrocyte vitamin E, GSH and catalase activity when compared to sedentary individuals[36]. We suggest that unexpected normal vitamin E levels in toll collectors may reflect a balance between oxidative stress and antioxidants in toll collectors.

Recently, some investigators proposed that the serum MDA and lipid peroxide levels with breath alkane output were good indices of lipid peroxidation in different clinical settings, such as smoking, HIV infection and inflammatory bowel disease[18]. Moreover, MDA was found to have a value when following disease progression and monitoring the efficacy of different treatments in neurodegenerative diseases[16]. In the present study, we suggest that serum MDA and nitrite+nitrate assays may be of considerable interest to measure oxidative stress among DE exposed workers. We also pointed out that with the inclusion of appropriate controls we could eliminate the effects of dietary intake on serum MDA, nitrite and nitrate levels. Food intake acutely and transiently increased serum NOx concentrations, an effect that was slightly attenuated if combined with alcoholic beverages. Chronic moderate alcohol consumption had no effect on the serum NOx concentration[37].

Since the serum levels of nitrite and nitrate are a complex estimate of DE exposure, probably both reflect the influence of direct NO exposure and an inflammatory process generated by the exposure, and further studies with a large number of subjects and with various assays for nitrite + nitrate levels as a marker of oxidative stress in DE exposure should be performed in high risk groups.

In conclusion, we demonstrated high serum MDA and nitrite+nitrate levels in toll collectors when compared with controls. We suggest that serum MDA levels may be used as a biological marker of oxidative stress related to DE exposure, so that preventive measures should be undertaken in manual tollbooths. Nevertheless, prospective controlled clinical studies are necessary to clarify a possible association among MDA, nitrite+nitrate levels and pulmonary diseases related to DE exposure.

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