Egyptian Journal of Occupational Medicine, 2011; 35 (1) : 83-101

STUDY OF MOTOR VEHICLE EXHAUST EXPOSURE, AS A RISK FOR GENOTOXICITY AND NEPHROTOXICITY AMONG PROFESSIONAL DRIVERS IN ZAGAZIG CITY, EGYPT.

By

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Abstract:

Objectives: vehicle exhaust consists of many toxic components and professional drivers are occupationally exposed to vehicle exhaust during their routine daily work. Hence, the aim of the present study is to evaluate genotoxic and nephrotoxic risk of vehicle exhaust in professional drivers in Zagazig city, Al-Sharkia Governorate, Egypt.

Subjects and Methods: A comparative cross sectional study was carried out on 33 professional drivers and 34 control office workers as non-exposed control group. A structured questionnaire was offered for all subjects through personal interview. Peripheral blood samples were collected from all participants for detection of chromosomal aberrations and measurement of blood lead level. Urine samples also were obtained from all participants for laboratory analysis to detect levels of B_2 – microglobulin, microalbumin and trans,trans- muconic acid.

Results: A significant increase was observed in the percentage of chromosomal aberrations in non smoker and smoker professional drivers when compared to their respective control groups. Also a significant increase in the mean values of B2 microglobulin and microalbumin was observed in studied professional drivers when compared to their control. The results showed that professional drivers have significant higher blood lead level and urinary trans,trans- muconic acid level as compared to control group, with positive correlation to the duration of exposure.

Conclusion: Thus the present study suggests that the induction of cytogenetic changes and renal damage might be due to the cumulative effect of smoking, lead toxicity,

benzene toxicity, leaded gasoline and prolonged duration of exposure to vehicle exhaust.
Key words: chromosomal aberration – B_2 microglobulin– microalbumin – Blood lead level – Trans, trans- muconic acid – benzene.

Abreviations: BLL: Blood lead level, t,t-MA: Trans- trans muconic acid

Competing interests:
The authors declare that there are no competing interests. This research paper was financed totally by the authors of the study.
Funding:
None.

Ethics issues:
Proposal acceptance was obtained from the Research Ethics Committee (REC) in Zagazig Faculty of Medicine. Moreover, All the participants were informed about the objectives of the study and their written consents were obtained.

Introduction

Air pollution in Egypt has been aggravated by a number of developments, such as, growth in the size of cities with increase in traffics, rapid economic development, industrialization, and high levels of energy consumption. Zagazig city is the capital of Sharkia governorate. It is 19 km^2 , located 80 km northeast of Cairo. It has a population of nearly one million habitants (El – Sayed et al., 2010).

Vehicular traffic density and automobile exhaust which consists of many toxic components are considered the major sources of air pollution particularly in urban cities. This air pollution results in serious health problems. Occupational exposure to these pollutants may furthermore lead to significant induction of cytogenetic damage in peripheral lymphocytes. Several studies in different countries have shown that the levels of DNA damage were higher among subjects more heavily exposed to air pollutants in vehicle exhaust (Sree Devi et al., 2009).

This observation has been made in different population categories, such as among police officers in Italy and Thailand, in residents in highly industrialized areas in Poland, workers exposed to traffic pollution in Florence and among bus drivers in Denmark. In all these studies the more exposed subjects had significant differences in DNA damage than those who were less exposed (Vineis and Husgafvel-Pursiainen, 2005).

Diesel and petrol constitutes a complex mixture of volatile flammable liquid hydrocarbons among them benzene (BZ), toluene (TOL), and xylene (XYL) are considered to be the most hazardous, predominantly BZ because of its

carcinogenic potency (Rekhadevi et al, 2011).

Some toxic compounds are present in gasoline and are emitted to the air when gasoline evaporates or passes through the engine as unburned fuel. Benzene, for example, is a component of gasoline. Motor vehicles emit small quantities of benzene in unburned fuel, or as vapor when gasoline evaporates. A significant amount of automotive benzene comes from the incomplete combustion of compounds in gasoline such as toluene and xylene that are chemically very similar to benzene. Like benzene itself, these compounds occur naturally in petroleum and become more concentrated when petroleum is refined to produce high octane gasoline.(U.S. Environmental Protection Agency 1994)

Concentration of benzene in ambient air has increased significantly in recent years due to increased road traffic. Vehicle exhaust is considered one of the major source of benzene in ambient air. There are some urinary metabolites that serve as indices of occupational or environmental exposure to benzene include phenol, hydroquinone, trans, trans- muconic acid, and S- phenyl mercapturic acid. Among these indices trans, trans-muconic acid concentration in urine is considered as a reliable biomarker which is relatively convenient to measure benzene exposure. Significant differences in levels of urinary trans, trans-muconic acid were found in drivers and petrol station workers when compared to a control group in Iran (Bahrami et al., 2007).

Lead is a well recognized environmental pollutant emerging from combustion in vehicles of petrol containing lead as antiknock additive. Lead toxicity is known to induce adverse health effects in multiple systems. Occupational exposure to lead was proved by some studies to be responsible for induction of genetic damage and nephrotoxicity (Vij ,2009).

Professional drivers are one of the groups who are long term occupationally exposed to lead from air pollution, especially when driving in heavy traffic congestion. There is limited published data about the health effects of chronic vehicle exhaust exposure among professional drivers in Egypt. So, this study was conducted to assess exposure to pollutants in vehicle exhaust among professional drivers in professional drivers in Zagazig city and to assess the risk for genotoxicity and nephrotoxicity in professional drivers chronically exposed to vehicle exhaust during their daily work.

Subjects and Methods

Study population:

A Comparative cross-sectional study was carried out on a simple random sample of drivers at 3 big bus stations on July 2010 in Zagazig city Al-Sharkia Governorate, Egypt.

Inclusion criteria of exposed group: drivers who are working regularly for at least five years in this occupation and are exposed to vehicle exhaust.

Exclusion criteria of exposed group: The drivers who reported medical illness of diabetes mellitus, chronic hepatic disease, chronic renal diseases and/ or who were on medication, and history of working in lead batteries, benzene stations or lived near a battery manufacturing factory.

Inclusion criteria for non exposed group: the administrative staff at Zagazig University who were not occupationally exposed to vehicle exhaust at their workplace and belonged to the same age group as the included cases.

The sample size was calculated by using Epi Info version 6.04; assuming the frequency of exposure among exposed group was 26%, at confidence level 95%, with test power 80%, and the ratio of exposed to non-exposed group equal 1:1. So the calculated sample size = 66 participants added to it 10 % for non response (7 participants). Then the number of cases & number of control=37 in each group.

The current study used different tools for data collection included;

I-Tailored questionnaire adapted from previous studies was offered to the selected drivers. The questionnaire included: personal data, occupational history, smoking habits, medical history, exposure to irradiation and chemotherapy.

Thirty seven drivers accepted to participate in this study and completed the questionnaire.

But four cases were excluded during laboratory analysis of their urine samples (pH<5.5, glucosuria, protienuria, urinary tract infection). So the final total number of the exposed group was 33 drivers. Only 34 office clerks accepted to be included in the present work.

II- Laboratory investigations:

Collection and preparation of samples for analysis:

1- Blood samples were collected by a trained medical assistant using sterile

syringes. 2 ml venous blood were collected into lithium heparin tubes from each one of exposed professional drivers and controls for evaluation of chromosomal aberrations in peripheral blood lymphocytes. Another 3 ml were collected in lead-free tube for measurement of blood lead level.

2-Urine samples were collected by participants using sterilized sample bottles that were given to them the night before. Ten milliliters early morning urine were collected for assessment of t,t-MA (Benzene metabolites), microalbumin levels and B2-microglobulin level in urine to assess renal affection. All the sample bottles were tightly covered, well labeled and refrigerated immediately then transferred to the laboratory and kept frozen until analyzed.

***1-Genotoxicity assessment*:**

Analysis of chromosomes aberrations was done to detect cytogenetic damage according to the method of **Verma, (1998).** Blood samples collected in lithium heparinized tubes, coded and sent to the laboratory. Lymphocyte cultures were set up by adding 0.5 ml whole blood to 4.5 ml RPMI 1640 medium supplemented with L-glutamine , 15% heat- inactivated fetal calf serum and 1% antibiotics (penicillin and streptomycin). Lymphocytes were stimulated by 1% phytohaemagglutinin (all obtained from Gibco laboratories) and incubated for 72 h at 37°C. Two cultures per subject were established. At 72 h of incubation, the cultures were harvested. Colcemid (10 ug/ml) was added to the culture and left for 30 min at 37°C then treated with Prewarmed hypotonic solution 0.075 M potassium chloride: KCL (5.59g/L) for 30 min at 37°C. Cells were centrifuged thereafter and a fresh, cold fixative solution (3:1 methanol: acetic acid) was added drop by drop slowly.

This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative solution and dropped onto clean slides from a height of about 40-60 cm. The slides were allowed to age for at least one day at 37°C.

Finally the slides were stained with 10% Giemsa in phosphate buffer (pH 6.8) for 10 min. Slides were scanned under a light microscope to locate the chromosome spreads, which were then examined under high power oil immersion x100 objective. At least 20 metaphases were examined. Chromosome analysis was done using an automated karyotyping system,

Fluorescence microscope (Olympus, BX40) and a computerized image analysis system (Morphostar Genetics workstation), Karyotyping was done according to the International System for Human Cytogenetic Nomenclature (Mitelman, 1995).

***2- Assay of blood lead level: (µg/dl)*:**

Blood lead level is measured according to **Evenson, (1999).** Measurement was achieved by atomic absorption using Buck atomic absorption spectrophotometer (model 2/0 UGP) adapted with heated graphite furnace with back ground correction; measurement was read at wave length 283 nm. The detection limit for blood lead was 10 µg/L.

The reference range for acceptable blood lead concentrations in healthy persons without excessive exposure to environmental sources of lead is less than 25 μg/dL for adults **(Wu, 2006).**

3- Assay for trans, trans-muconic acid in urine(t,t-MA):

Assays of t,t-MA concentrations in the urine samples was done according to the method of (Boogaard and Van Sitter, 1996). To improve the recovery, urinary samples were brought to pH 10 by the addition of 35% sodium hydroxide aqueous solution.

Samples were centrifuged (2.000 rpm for ten minutes) to separate suspended materials and 1 ml was subsequently passed through a SAX column (100–200 mesh, 1 cm diameter, 10 cm height), which had been previously conditioned with 3 ml acetonitrile and 3 ml deionized water. After washing with 3 ml of 1% acetic acid, t,t-MA was eluted from the cartridge with 4 ml 10% acetic acid. Twenty micro liters of this solution was injected onto a HPLC column (hypersil (125 x 4 mm I.D., 5 μm Particle size). The mobile phase consisted of 1% (v/v) acetic acid, 10% (v/v) methanol, and 89% (v/v) water. The flow rate was 1 ml/min. The effluent was monitored with a UV detector which was set at 259 nm. The standard trans, trans- muconic acid (98%) was obtained from Aldrich- Chemistry M9003-IG-07319CH, Sigma. The analyte and the standard were eluted at 14-15 min, respectively. The detection limit was 0.05 mg/L. Urine t,t-MA concentration was reported as mg/g creatinine.

The reference range of trans, trans-muconic acid in non-exposed subjects was 0.03 to 0.26 mg/g of creatinine (De Paula et al.,2003).

4- Measurement of microalbuminuria:

Urinary microalbumin concentration was determined by immuno-turbidometry

Latex method according to Cambiaso et al. (1988) using Bio-Systems kit, Costa Brava 30, Barcelona (Spain). Albumin in the urine sample causes agglutination of the latex particles coated with anti-human albumin. The agglutination of the particles is proportional to the albumin concentration and can be measured by turbidimetric with a detection limit of 0.9 mg/l albumin.

5- Measurement of B2 micro- globulinuria:

Excretion of urinary B_2-microglobulin was assessed by chemiluminescent technique using Siemens Medical Solutions Diagnostics (Glyn Rhonwy, United Kingdom) via Immulite 2000 Analyzer, diagnostic products corporation (DPC), Los Angeles, USA according to Moriguchi et al, (2003).

6- Statistical analysis:

All statistical analysis was performed using computerized statistical package of social science (SPSS) version 8.0 soft-ware **(Norusis, 1997).** Qualitative data were compared using Chi- square test, while quantitative data were compared using student's t-test, and Spearman correlation test was used to evaluate the association between two continuous variables. LSD test was also used in statistical analysis. Differences was considered significant at $p < 0.05$.

IV-Ethical consideration:

The ethical committee of the Zagazig faculty of Medicine approved the study protocol. All the participants were informed about the objectives of the study and their written consents were obtained.

Results

A total of 33 professional drivers and 34 control individuals participated in this study. Their mean age was 52.5±7.6 and 50.8±6.7 respectively. Statistical difference was not found between studied drivers group and control group in their mean age, mean years of work and daily working hours. There was significant statistically difference between studied drivers and control group in number of cigarettes consumed per day Table (1).

Regarding genotoxicity assessment, the results have shown a statistically significant increased chromosomal aberrations in peripheral lymphocytes of exposed professional drivers when compared to controls ($p<0.05$). Among studied drivers chromosomal aberrations appeared in the form of chromosomal breaks (8.78%), chromosomal gap (4.54%) and fragments (4.4%) Hyperploidy was the least frequently observed (1.06%). In control group chromosomal aberrations were in

the form of breaks only in 4.71%. The results of chromosomal aberrations were also analyzed according to the duration of occupational exposure to vehicle exhaust in drivers exposed for <25 years, 25-35 years and >35 years with significant statistically difference between groups appeared with increased duration of exposure, $p<0.05$ Table(2)

Nephrotoxicity was also assessed among studied drivers and control group through measuring B_2-microglobulin (μg/l) and microalbumin (μg Alb./mg Cr.). Mean values of B_2-microglobulin among both groups were 221.5±37.4 and 103.0±15.5 (μg/l) respectively with statistically significant difference between them ($p<0.05$).Regarding mean values of microalbumin among both groups they were 30.3±5.6 and 11.1±2.5 (μg Alb./ mg Cr.) respectively which showed also a statistically significant difference between both groups. When the results of both markers were allied to years of occupational exposure to vehicle exhaust among studied drivers, significant statistically difference appeared between inter groups of studied professional drivers regarding to exposure duration as illustrated in Table(2).

The results were further analyzed according to the blood lead level (μg/dl) and the urinary level of t,t-MA (mg/g creatinine). The results showed high statistically significant difference in mean value of both markers between the studied drivers and control group $p<0.001$. Among studied professional drivers there were statistically significant differences between those exposed 25-35 years compared to drivers exposed <25years in mean value of blood lead level, 38.9±15.4 and 31.1±11.2 μg/dl respectively. The results showed statistically significant difference between drivers exposed >35years with mean value 42.7±7.2 μg/dl compared to those exposed 25-35years. There was also significant positive correlation between years of exposure and both markers among studied professional drivers Table (3).

On studying smoking effect on studied population regarding chromosomal aberrations, there was significant difference $p<0.05$ when comparing both smoking and non smoking professional drivers with the respective control group. Also, when comparing smokers with the respective non smoker among both studied drivers and control there was significance difference in between $p<0.05$ Table (4).

The results of this study proved a significant effect of occupational exposure to both lead and benzene from vehicle

exhaust on chromosomal aberrations. This results appeared on studying the increase in blood lead level > 40 μg/dl and urinary trans, trans-muconic acid (t,t-MA) > 2 mg/g creatinine, and their relationship with chromosomal aberrations among studied professional drivers. On the other hand only occupational exposure to lead from vehicle exhaust was statistically associated with nephrotoxicty among studied professional drivers, while the increase in urinary trans, trans- muconic acid (t,t-MA) showed no significant association with nephrotoxicity among them Table (5&6).

Table (1): Age, occupational characteristics and smoking habits of the studied population

Variables	Control group (n=34)		Professional drivers (n=33)	
	N	%	N	%
Age (years)				
Mean years	50.8±6.7		52.5±7.6	
<45 yrs	8	23.53%	6	18.18%
45-55 yrs	20	58.82%	18	54.54%
>55 yrs	6	17.64%	9	27.27%
Duration of work				
Mean years	24.6±8.2		27.7±9.6	
<25 yrs	6	17.64%	11	33.33%
25-35 yrs	17	50%	18	54.54%
>35 y	11	23.35%	4	12.12%
Daily Working hours				
Mean hours per day	8.7±1.1		10.2±2.4	
Smoking habits				
Duration of smoking per years	24.0±8.5		29.6±8.5	
Number of cigarettes per day	11.0±4.6		19.4±9.3*	

*= significant compared with control group ($p<0.05$)

Table (2): Chromosomal aberrations & B_2-microglobulin and microalbumin regarding period of exposure among studied professional drivers compared to control group.

Variables / Groups		Control Office clerks N=34	Professional drivers (<25 yrs) N=11	Professional drivers (25-35 yrs) N=18	Professional drivers (>35 yrs) N=4	Total number of drivers N=33
Chromosomal aberrations	Analyzed metaphases	680	220	360	80	660
	Breaks	32 4.71%	12 5.45%	38 10.6%	8 10.0%	58 8.78%*
	Gap	0 0.00%	10 4.54%	15 4.17%	5 6.25%	30 4.54%*
	Fragments	0 0.00%	4 1.8%	19 5.27%	6 7. 5%	29 4.4%*
	Hyperploidy	0 0.00%	0 0.00%	7 1.44%	0 0.00%	7 1.06%*
	Total	32 4.71%	26 11.8%	79 21.9%≠	19 23.75% †	122 18.5%*
Nephrotoxicity	B_2microglob-ulin (μg/l)	103.0±15.5	**202.5**±33.6*	**209.3**±16.4*≠	**262.2**±33.9*≠†	221.5±37.4*
	Microalbumin (μg Alb./mg Cr.)	11.1±2.5	28.2±4.1*	29.95±5.7*	34.4±4.8*≠†	30.3±5.6*

*= significant compared with control group (p<0.05)
≠P= <0.05 significant when compared with <25 years
†P= <0.05 significant when compared with 25-35 years

Table (3): Blood lead level & urinary t, t-MA level regarding period of exposure among studied professional drivers compared to control group.

Groups / parameters	Control Office clerks N=34	Professional drivers (<25 yrs) N=11	Professional drivers (25-35 yrs) N=18	Professional drivers (>35 yrs) N=4	P
Blood lead level(*μ*g/dl)	21.1±4.1	31.1±11.2*	38.9±15.4*≠	42.7±7.2*≠†	<0.001
t, t-MA level (mg/g creatinine)	0.24±0.06	1.96±1.1*	2. 37±1.4*	2.15±1.6*	<0.001

*= significant compared with control group p<0.001
≠P= <0.05 significant when compared with <25 years
†P= <0.05 significant when compared with 25-35 years
Correlation was done for the duration of exposure among studied drivers:
For blood Lead level: r = 0.04 &P <0.05
For t,t-MA level :r = 0.35 & P < 0.05

Table (4):Effect of smoking on chromosomal aberrations among professional drivers and respective control.

Studied groups		Control group		Professional drivers	
Smoking habits		Non- smoker N=9	Smoker N=25	Non-smoker N=5	Smoker N=28
chromosomal aberrations	Analyzed metaphases	180	500	100	560
	Total CAs (%)	5 2.77%	27 5.4%†	13 13%*	109 19.46%†*

CAs= chromosomal Aberrations
*= significant difference (p<0.05) when comparing professional drivers with the respective control group
†= significant difference (p<0.05) when comparing smokers with the respective non smokers group

Table (5): Effect of increased blood lead levels on chromosomal aberrations& nephrotoxicity, among studied drivers.

Blood. Lead level (μg/dl) Variables		Blood Lead level < 40 μg/dl N=15	Blood. Lead level ≥ 40 μg/dl N=18	p
Chromosomal aberrations	Analyzed metaphases	300	360	
	Breaks	21 7.0%	37 10.2%	<0.05
	Gap	15 5.0%	15 4.17%	<0.06
	Fragments	10 3.3%	19 5.28%	<0.2
	Hyperploidy	1 0.3%	6 1.7%	<0.19
	Total	46 15.3%	76 21.11%	<0.001
Nephrotoxicity	B_2micro globulin (μg/l)	204.3±17.2	238.6±44	<0.05
	Micro-albumin (μg Alb./mg Cr.)	26.9±4.2	33.8±4.6	<0.05

Table (6): Effect of increased urinary trans, trans-muconic acid (t,t-MA) levels on chromosomal aberrations & nephrotoxicity among studied drivers.

t,t-MA (mg/b creat.) Variables		t,t-M A level < 2 mg/g creatinine) N =17	t,t-M A level ≥ 2 mg/g creatinine N =16	P
Chromosomal aberrations	Analyzed metaphases	340	320	
	Breaks	9 2.6%	28 8.75%	<0.05
	Gap	3 0.88%	9 2.8%	<0.06
	Fragments	3 0.88%	11 3.4%	<0.05
	Hyperploidy	0 0.0%	2 0.56%	<0.06
	Total	15 4.4%	76 18.75%	<0.001
Nephrotoxicity	B_2micro globulin (μg/l)	226.2±41	215.2±32	<0.30
	Micro-albumin (μg Alb./mg Cr.)	30.6±5.1	29.99±6.3	<0.55

Discussion

Chromosomal aberrations in peripheral blood lymphocytes have been used for decades for the surveillance of exposure to known or potential mutagens and carcinogens. In general, it is accepted that chromosomal mutations are causal events in the development of cancers and increased cytogenetic damage may be an indication of an enhanced cancer risk. Elevated level of chromosomal aberrations in peripheral blood lymphocytes is a probable indicator of early phase of carcinogenesis and is considered as an early marker of cancer risk (Boffetta et al., 2007; Sree Devi et al., 2009).

The present results showed a significant increase in the percentage of different forms of chromosomal aberrations (CAs) in professional drivers when compared to the controls, this is consistent with the results of earlier studies done on bus drivers in Copenhagen, Denmark and, taxi drivers in Ankara, Turkey, and traffic policemen in Hyderabad, India which reported a significant increase in chromosomal aberrations among individuals occupationally exposed to petrol and vehicle exhausts and fumes. However, some studies did not prove this association. This may be attributed to type of occupation, levels of air pollution, traffic congestion, vehicle maintenance and quality of the fuel (Kamboj and Sambyal, 2006; Sree Devi et al., 2009).

When studying the exposure duration effect, the results of the present study revealed increase in chromosomal aberrations and genetic damage with increasing years of exposure. This is concomitant with earlier studies on persons exposed to pollutants from petrol and vehicle exhausts, reporting a positive correlation between higher incidence of chromosomal aberrations and increasing exposure duration (Kamboj and Sambyal ,2006; Sree Devi et al., 2009).

Smoking has enhancement effect on chromosomal aberrations over subjects in this study both drivers and controls. There was a significant increase in chromosomal aberrations among smoking drivers over non smokers, however there was still significant increase in chromosomal aberrations among non smoking drivers over non smoking controls. The result of the present study regarding smoking is consistent with other studies proved that smoking has an synergistic effect to vehicle exhaust in producing cytogenic damage (Sree Devi et al., 2009).

Vehicular traffic was the single largest source of environmental lead pollution in the world until its use in gasoline was stopped (Vij, 2009). The Lead Group Inc. reported that Egypt is one of the countries where leaded petrol is still sold for road use (Taylor , 2010). In spite of stoppage of using leaded gasoline in great Cairo (the Egyptian Capital) but it is still in use by most of the Egyptian governorates (Sharaf et al., 2008).

The results in the present study showed significant increase in blood lead level among studied drivers compared to the control group, and these levels were exceeding the normal accepted values in adults (25 μg/dl) as stated by Wu (2006). This increase is correlated with duration of work as lead is known to have a long half-life and accumulates in human body over a lifetime. These results are consistent with earlier studies which reported higher blood lead level in traffic policemen exposed to vehicle exhausts than the non-exposed persons (Mortada et al., 2001; Agha et al., 2005). It was also noticed that the mean of blood lead level among both studied drivers and control was higher than that reported in other studies and this may be attributed to the high level of usage of leaded petrol in Egypt till now as it is much cheaper than unleaded petrol in addition to the contribution of industrial emissions while lacking environmental control measures.

The International Agency for Research on Cancer has classified lead as possible human carcinogen (group 2B) and its inorganic compounds as probable human carcinogens (group 2A) (Garcia-Leston et al., 2010).

Data now implicates low- level exposures and blood lead levels previously considered normal as causative factors in cognitive dysfunction, neurobehavioral disorders, renal impairment, chromosomal aberrations, somatic and germ cell mutations and genotoxicity (Vij ,2009).

Lead induced chromosomal aberrations was proved by previous studies that revealed a significant increase in frequency of chromosomal aberrations in workers exposed to lead in different occupations than controls (Shaik and Jamil ,2009; Grover et al., 2010). This was obvious in this study where drivers with high blood lead level $\geq$ 40 μg/dl had a significant increase in chromosomal aberrations than those with blood lead level $<$ 40 μg/dl.

Effect of vehicle exhaust exposure on renal system was assessed in this study

through measuring urinary B_2microglobulin and microalbumin. Both markers showed significant increase among studied drivers compared to control group.

This increase was also correlated with duration of vehicle exhaust exposure. Significant difference was also noticed between drivers has blood lead level ≥ 40 μg/dl and those had blood lead level < 40 μg/dl. This result is in accordance with the results of earlier studies which revealed significant increase in urinary B_2microglobulin and/or microalbumin as markers of nephrotoxicty due to occupational exposure to lead (Mortada et al., 2001; Anetor, 2002).

Chronic occupational exposure to lead is characterized by glomerular and tubulointerstitial changes. These changes are irreversible and may end in renal failure. These changes is accompanied by increased excretion of B_2-microglobulin and albumin which are considered as early and sensitive indicator to predict for renal toxicity from lead exposure (Loghman- Adham , 2008).

Other parameter for assessment of exposure to traffic-associated pollutants is measuring t,t-MA which is accepted as a useful monitoring tool for benzene. Benzene is mainly emitted from motor vehicle traffic and it is a well-known carcinogen that induces chromosomal instability, including chromosomal aberration and aneuploidy (Bahrami et al., 2007; Kim et al., 2010).

The present study results showed a significant increase in t,t-MA in urine of studied drivers compared to control group. These levels were higher than the accepted reference values for t,t-MA (0.03 to 0.26 mg/g) as stated by (De Paula et al., 2003). Moreover, this increase was correlated with duration of exposure. Earlier studies on individual occupationally exposed to benzene revealed significant increase in urinary t,t-MA compared to their control (Bahrami et al., 2007; Rekhadevi et al., 2011).

The mean urinary t, t-MA level in the present study was higher than other studies done in Iran (0.31 mg/g creatinine) among taxi drivers and (2.64 mg/g creatinine) in petrol stations' workers, and Tunis (0.11 mg/g creatinine) but lower than that done in Bangkok (4mg/g creatinine). This is an indication of higher exposure to ambient air benzene in our country however unfortunately it was not feasible in this study to do environmental monitoring and measure ambient air levels due to lack of instruments (Bahrami et al., 2007).

Studying the effect of benzene on inducing chromosomal aberrations among studied drivers in the present study showed a significant increase in chromosomal aberrations among drivers with t,t-MA level ≥ 2 mg/g creatinine than those with t,t-M A level < 2 mg/g creatinine. These results are consistent with other studies proved that exposure to high levels of benzene has a genotoxic effect (Angelini et al., 2011; Fracasso et al., 2010).

Moreover, the current study clearly showed that professional drivers has elevated BLL and t,t-MA indicating that those drivers were exposed to higher levels of lead and benzene exposure in comparison to control , this co-exposure resulted in augmentation of the genotoxic effect of both toxins as reported previously by Al-Faisal et al., (2010). They found that lead exposure in petrol station workers in Baghdad, Iraq had resulted in an increased percent of chromosomal aberrations indicating that, both toxins have synergistic genotoxic effect.

Conclusion

Professional drivers in Zagazig city (one of traffic crowded cities of Egypt) were occupationally exposed to several pollutants in vehicle exhaust that results in cytogenic damage and kidney damage. These effects were enhanced by duration of exposure and smoking. Environmental monitoring to assess levels of pollutants in ambient air is required in further studies. Restricted policies to maintain old vehicles and innovation of solutions for traffic congestion and raising awareness regarding ambient air pollution are recommended to help in providing safety for individuals in such outdoor occupation.

Acknowledgment

Great thanks and gratefulness to Dr. Maha El-Deeb and D. Felibs Hanna, Fellows of clinical Pathology at Central Laboratory, Faculty of Veterinary Medicine, Zagazig University for helping in performing estimation of t,t-MA levels and blood lead levels.

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