



Chromosomal Abnormalities among Petrol Station Workers Occupationally Exposed to Benzene

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Authors' contributions

This work was carried out in collaboration between all authors. Author NMEM designed the study, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors NMR, HSK shared in writing the manuscript, managed the analyses of the study and literature searches. Author FEH performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

It is known that the vast majority of malignancies are the result of genetic and environmental interactions. Fuel (diesel and petrol) constitutes a complex mixture of benzene, toluene and xylene and other monocyclic, aromatic, aliphatic and polycyclic aromatic hydrocarbons; benzene being the most hazardous because of its carcinogenic potency. This study aims to evaluate the genotoxic effects of occupational exposure to benzene through cytogenetic analysis at low levels of exposure also to investigate the possibility of liver and kidney affections. The studied group comprised 40 workers from 3 different petrol stations occupationally exposed to benzene and 20 control subjects. The two studied groups were matched regarding age and sex. Results show significant difference between exposed workers and control group in chromosomal abnormalities regarding mitotic index, gap, isogap and break. Regarding period of exposure, the frequency of different types of chromosomal aberrations was relatively high during first few years of exposure and decrease with

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increasing duration. Regarding liver function tests, levels were within normal ranges but higher in workers with statistically significant difference between exposed workers and control group and they were significantly correlated with the duration of exposure (p value= .05). Occupational exposure to mutagenic and carcinogenic agents creates a significant impact on the health status of gasoline station attendants. Identification and prevention of possible health problems related to such exposure would help in maintaining good health of workers; prevent reduction in working capacity and loss of working hours besides reducing the cost of medical care for affected workers. Further studies are needed to assess DNA status in the lymphocytes that might be a predictor of future cancer risks and might help to prevent further deterioration in the health of these workers.

Keywords: Benzene; gas stations; chromosomal aberrations; preventive measures.

1. INTRODUCTION

Fuel (diesel and petrol) constitutes a complex mixture of benzene (BZ), toluene (TOL), and xylene (XYL) and other monocyclic, aromatic, aliphatic and polycyclic aromatic hydrocarbons; BZ, TOL and XYL are considered to be the most hazardous, predominantly BZ because of its carcinogenic potency. Exposure to these compounds may have an impact on the health of the exposed subjects [1].

The volatile nature of petrol makes it readily available in the atmosphere whenever it is dispensed, especially at petrol filling stations. According to the IARC, exposure to gasoline vapors is stated as carcinogenic to humans [2,3]. Petrol vapor is not safe even when inhaled for a brief period of time during fuelling vehicles which puts the gas station attendants at more risk by virtue of their occupational exposure [4].

Egypt has a large population, estimated to be 84,629 in 2013 according to CAPMAS with 42.8% living in urban areas [5]. Cairo is the most crowded area with high traffic overload that contribute to air pollution. Higher exposures to engine exhausts may occur in some occupations such as petrol station attendants.

Prolonged exposure to benzene causes various effects on human body especially myelotoxicity, genotoxicity and its carcinogenic actions. Other effects on various organs e.g. the central nervous system, the endocrine and immune system [6].

To evaluate the impact of occupational exposure on health, it is essential to identify the effects of exposure through epidemiological studies [7]. Analytical epidemiologic methods employ various biomarkers, rather than disease, to assess the risk of environmental exposure. The prevalence of these biomarkers in test subjects indicated a

greater amount of genotoxic exposure and a greater risk towards the development of diseases. One such biomarker of abnormal cell division involving chromosomal breakage, deletions [8].

Today, chromosomal aberrations (CAs) in human peripheral lymphocytes are recognized as a valuable biomarker of effect, probably the only one which has been internationally standardized and validated [9].

CAs could result from exposure of cells to PAHs. CAs, either unstable (e.g. breaks) or more serious stable translocations, which are conserved in the genome and may lead to changes in the expression of various genes, including oncogenes, may be formed by two different mechanisms. The production of ROS as a result of PAH metabolism and their interaction with DNA may induce strand breaks [10].

If double strand breaks are formed and then processed by DNA repair mechanisms (homologous repair or non-homologous end-joining repair), CAs may result. Another pathway leading to CAs is initiated by PAH-DNA adducts. The adducts did not form double strand breaks directly, but as by-products of repair mechanisms [11].

Generally, DEPs incline to form spherical, respirable aggregates with approximately 80–90% of them are in the aerodynamic diameter range of 0.05–1.0 μ m with a mean particle diameter of about 0.2 μ m [12].

Genotoxicity is thought to be crucial for the development of malignancy. Numerous *in vivo* and *in vitro* studies have suggested that size distribution, surface properties, and adsorbed chemicals are important criteria for the genotoxicity of DEPs [13,14].

Of many chemicals adsorbed to respirable particles carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) are regarded as one of the most important [15]. c-PAHs are metabolized into reactive intermediates that may bind to DNA and form PAH-DNA adducts, thus causing mutations and increasing cancer risk [16,17].

2. MATERIALS AND METHODS

This study is a case-control study. It was carried out on a sample of workers exposed to benzene at petrol stations. The sample was determined by convenience and consisted of all gas station workers who agreed to participate in the study after the gas station proprietary permission. Three gas stations located in El Manial, El Maadi and El Haram was chosen so as to represent crowded areas and one on the high way. Forty workers from 3 petrol stations and 20 control subjects were enrolled in this study. They were all males with an age range of 22- 72 years old and a median of 38.1 ± 12.1 . The gas station workers routinely work for 6 days a week, during 8 hours or more per day. The median duration of their employment in the gas stations was 9 years (range 1-50 years). 17 of workers (42.5%) were smokers while 23 (57.5%) did not smoke.

The studied groups were subjected to full history taking, including standard demographic data (age, marital status, etc) as well as past medical history, occupational history (working hours/ day, years of exposure, use of personnel protective measures, extra working hours).

2.1 Laboratory Investigations

Seven ml of venous blood samples were collected from each worker and from the controls.

Five ml of blood were left to clot, centrifuged for serum preparation and chemical analysis which was performed using the photometer PM 750 for measurement of SGPT, SGOT (normal values up to 38 U/L), serum creatinine (normal value = 0.6 - 1.2 mg%) and blood urea (normal value = 10-40 mg%).

2.1.1 Cytogenetic analysis

Structural and numerical chromosomal aberrations (CA) in peripheral blood lymphocytes were done using the G-banding technique.

Venous blood sample (3 ml) was collected once from all the exposed and control group subjects using heparinized syringes. Blood samples were coded to avoid possible bias. The samples were transported to the laboratory and were processed within 2 h after collection.

The CA analysis was conducted following a standard protocol with slight modifications. Half ml heparinized whole blood was cultured in RPMI with L-glutamine medium supplemented with 20% fetal bovine serum (FBS) (Euroclone, Europe), 200 ul phytohaemagglutinin, 100 ul penicillin and streptomycin, 100 ul antimycotic and 25 ul preserved heparin. Each culture was incubated in 5% CO₂ incubator at 37°C for 72 hours. Metaphases were obtained by adding colcemide to the cultures at a final concentration 0.4 ug/ml 2 hours before harvesting. The cells were collected by centrifugation, re-suspended in a pre-warmed hypotonic solution (0.075 M KCl) for 30 min at 37°C and fixed in acetic acid-methanol (1:3 v/v). Chromosome preparations were stained using 4% Giemsa stain. The slides were analyzed using the high power of the light microscope and 25 metaphases were screened per each individual. Cells with 46 chromosomes were scored for CA. The analysis of CA included chromatid and chromosome breaks, chromatid gap, chromatid deletions, chromatid rings, dicentrics, centromere separation and endomitosis.

2.1.1.1 Air sampling

The average benzene concentration measured in environmental air of the work place of all studied stations was 97.56 ± 88.12 ng/L (range: 4.69 – 260.86 ng/L) as measured by the electronic nose [18].

2.1.1.2 Statistical methods

Data were collected, checked, revised and entered the computer. Data were analyzed by SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 20. Excel computer program was used to tabulate the results and represent it graphically.

Qualitative variables were expressed as count and percent. The significant difference in the distribution of the qualitative variables were tested by using Chi square test of distribution at $p \leq 0.05$.

Quantitative variables from normal distribution were expressed as mean±S.D. The significant difference between the exposed and control groups were tested by using independent t-test at $p \leq 0.001$.

Pearson correlation coefficient was calculated to measure the power and direction of the relationship between the quantitative variables at $p \leq 0.05$ [19].

3. RESULTS

The studied group comprised 40 workers occupationally exposed to benzene in 3 different petrol stations with different levels of exposure and 20 control subjects away from such exposure. The workers were all males with age ranging from 22 to 72 years with a mean value of 38.10 ± 12.11 . The duration of exposure ranged from 1 to 50 years with a mean of 12.90 ± 11.52 years. The age of the control group ranged from 22- 66 years with a mean of 42.85 ± 13.40 .

17 subjects among exposed group (42.50%) were cigarette smokers versus 8 subjects (40.00%) from the control group. Twelve subjects (30%) of the exposed group had duration of employment less than 5 years with a mean value of (2.16 ± 0.46) , ten subjects (25%) had a duration of employment from 5 – 15 years with a mean value of (7.50 ± 0.65) while 18 subjects (45%) had been employed for more than 15 years with mean value of (23.05 ± 2.27) .

No person mentioned has acute diseases. As for frequency of chronic diseases, there was no difference among the groups. The chronic diseases observed in the case group were hypertension (n=5), diabetes (n=2), in controls there were hypertension (n=3), diabetes (n=5)

Table 1 shows the demographic characteristics of the studied groups which revealed that there was no statistically significant difference between the exposed workers and control group as regards the age. It shows also the pattern of the duration of exposure among exposed workers.

Table 2 revealed that there was significant difference between exposed workers and control group in chromosomal abnormalities regarding mitotic index which show higher levels among

controls indicating defective cell division in exposed workers, also significant difference was found in total aberrations without gap. Regarding different types of aberrations significant difference was found in gap, isogap and break.

Regarding period of exposure, Table 3 shows that the frequency of different types of chromosomal aberrations was relatively high during first few years of exposure and decrease with increasing duration; this finding could be explained by the so called "healthy worker effect" Páldy and colleagues in 1987 mentioned that the more healthy workers remain employed and tolerate this toxic exposure [20].

On studying smoking effect on the studied population regarding chromosomal abnormalities Table 4 shows significant difference ($p < 0.05$) between smokers of exposed group and those of control group regarding chromosomal abnormalities in total aberrations with gap and different types of aberrations.

Hepatotoxicity was also assessed among studied workers and control group through measuring ALT and AST levels. Results revealed that in workers mean ALT levels was 34.875 ± 19.499 , mean AST levels was 40.475 ± 16.048 , while in control group mean ALT levels was 21.950 ± 5.306 , mean AST levels was 31.650 ± 6.706 . Although levels within normal ranges but higher in workers with statistically significant difference between exposed workers and control group ($p < 0.05$) (Table 5 and Graph 1). In exposed workers levels were significantly correlated with the duration of exposure (p value ≤ 0.05).

Kidney function tests were within normal ranges with no statistically significant difference between exposed workers and control groups (Table 5 and Graph 1).

Table 6, Graph 2 illustrate the distribution of the investigated group regarding clinical manifestations. There was statistically significant difference between exposed workers and control group in most of the manifestations (asthma, chronic chest infection, amnesia, headache, dizziness, leg pain, varicosities, lower limb edema, skin allergy and blurring of vision).

Table 1. Demographic characteristics of the studied group

		Number	Range		Mean±S.D	P-value
			Min.	Max.		
Age	Exposed workers	40	22	72	38.1±12.11	0.494
	Controls	20	22	66	42.85±13.4	
Duration of exposure	< 5 years	12	1	5	2.16±0.46	0.000**
	5 - < 15 years	10	6	12	7.50±0.65	
	≥15 years	18	15	50	23.05±2.27	

All values are represented as mean±S.D, *= there is a significant difference by using independent t-test at p-value<0.05, **= highly significant difference by using independent t-test at p-value<0.001

Table 2. Comparison between exposed group and controls in chromosomal abnormalities

Variables	Groups	Exposed workers			Controls		P-value	
		Number		Mean±SD	Number			Mean±SD
		Min	Max		Min	Max		
Chromosomal Abnormalities Types of aberrations	Mitotic index	1	27	10.70±9.757	3	44	21.45±13.04	0.001**
	Total aberrations with gap	0	15	6.50±3.479	3	8	5.15±1.53	0.126
	Total aberrations without gap	0	5	1.15±1.189	1	4	2.05±1.28	0.014*
	Gap	0	10	4.83±2.620	1	7	3.00±1.62	0.002*
	Isogap	0	2	0.50±0.716	0	1	0.10±0.31	0.022*
	Breaks	0	2	0.53±0.640	0	4	1.30±1.13	0.003*
	Deletions	0	5	0.38±0.868	0	1	0.30±0.47	0.889
	Fragments	0	1	0.20±0.405	0	1	0.10±0.31	0.331
Centromeric attenuation	0	2	0.05±0.316	0	2	0.25±0.64	0.073	
All aberrations	0	14	6.48±3.419	3	8	5.05±1.504	0.078	

All values are represented as mean±S.D, *= significant difference at p-value<0.05, **= highly significant difference at p-value<0.001

Table 3. Types of chromosomal aberrations among exposed workers regarding period of exposure

Variables	Groups	Workers ≤ 5 years		Workers 5- 15 years		Workers ≥ 15 years		P- value	
Chromosomal aberrations	Isogap	1, 2	6	54.6%	6	37.5%	3	23.1%	0.642
	Breaks	1,2	5	45.5%	7	43.8%	6	46.2%	0.999
	Deletions	1, 5	3	27.3%	5	31.3%	3	23.1%	0.807
	Fragments	1	1	9.1%	4	25.0%	3	23.1%	0.564
	Centromeric attenuation	2	1	9.1%	0	0.0%	0	0.0%	0.259

4. DISCUSSION

It is known that the vast majority of malignancies are the result of genetic and environmental interactions. Benzene is classified as a group 1 carcinogen by the International Agency for Research on Cancer and is listed by the World

Health Organization as a top priority compound [21].

Benzene, even at low concentrations, is a regular myelotoxic [22], leukemogenic [23] and carcinogen agent [24].

Table 4. Comparison between smokers of exposed group and those of control group regarding chromosomal abnormalities

	Group	Mean±S.D	t- test	P-value
Mitotic index	Exposed [17]	0.012±0.009	0.911	0.386
	Control [8]	0.017±0.012		
Total aberrations with gap	Exposed	6.117±3.425	0.986	0.036*
	control	4.875±1.246		
Total aberrations without gap	Exposed	1.117±1.166	1.439	0.634
	control	1.875±1.356		
Types of aberrations	Exposed	0.244±0.137	0.986	0.036*
	control	0.195±0.049		

All values are represented as mean±S.D, *= significant difference at p-value<0.05

Table 5. Comparison between exposed and control groups in laboratory investigations

	Group	Mean±S.D	t- test	p-value
ALT (U/L)	Workers	34.9±19.5	2.90	0.00*
	Controls	21.95±5.3		
AST (U/L)	Workers	40.5±16.0	2.35	0.00*
	Controls	31.7±6.7		
Creatinine (mg%)	Workers	0.94±0.18	0.11	0.136
	Controls	0.95±0.14		
Urea (mg%)	Workers	27.7±6.2	1.93	0.687
	Controls	31.0±6.4		

All values are represented as mean±S.D, *= significant difference at p-value<0.05

The study was performed in 3 petrol stations and the mean measured benzene air level is within normal range; although several changes have been recorded, the most important being the chromosomal abnormalities which might denote pre-carcinogenic effects.

Concomitantly, evidence suggests that chromosomal instability is characteristic of dysplasias and many premalignant conditions and specific chromosomal aberrations appear to be associated with many types of cancer. These abnormalities can activate oncogenes or result in the loss of tumor suppressor genes [25]. Zhang and colleagues in 2002, 2005 and 2011 detected specific chromosomal aneuploidies and aberrations in the blood cells of benzene-related leukemia patients as well as in healthy benzene-exposed workers, suggesting that these abnormalities preceded and may be a potential mechanism underlying benzene-induced leukemia [26-28].

In the case of aromatic hydrocarbons, the monitoring of environmental levels, knowledge of their routes of entry into the body and their metabolism, as well as the early evaluation of their biological effects are of great importance for the prevention of the development of certain cancers [23]. When inhaled, these organic

compounds are not only absorbed through the lungs but can also be absorbed through the skin [29]. Several components of this group of substances are capable of reacting with DNA, directly or after metabolic transformation, becoming potential mutagens and carcinogens [22,23].

When we compared our results with others in literature, we verified similar findings with several other studies that have been carried out to determine the chromosomal aberrations (CA) in benzene exposed workers.

Carere and colleagues in 1995; Tawn and Whitehouse in 2001 stated that in lymphocytes and bone marrow cells of workers exposed to benzene, numerical and structural chromosomal changes were observed [30,31].

In 1994, Tompa and his colleagues found that the frequencies of chromosomal aberrations were significantly higher in exposed workers than in controls thus providing evidence for the clastogenic effects of benzene. However, they didn't find correlation between aberration frequencies and the duration of prior exposure to benzene. When the benzene concentrations were brought down, there was a concomitant decrease in the frequencies of chromosomal

aberrations, but values still higher than in the controls [32]. Also, Biro´ and colleagues in 2002 found higher frequencies of CA in exposed workers [33]. Similarly, results of Hammam and colleagues in 2011 showed significant increase in the percentage of different forms of CAs in professional drivers exposed to motor vehicle exhaust. CAs appeared in the form of breaks, gaps and fragments. When they relate these to the duration of exposure they found statistically significant difference between groups with increased duration of exposure [34].

Zhang and colleagues in 2005, Santiago and colleagues in 2014 found that benzene exposure had been strongly associated with increased chromosomal abnormalities in the lymphocytes in individuals without diseases [27,35].

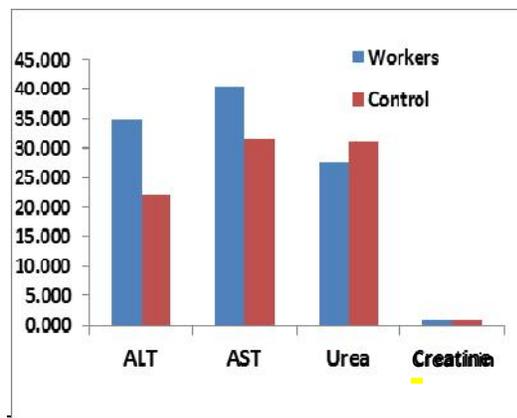
Topinka and colleagues in 2007 stated that one of the major findings of their study is that there was direct association between personal exposure to c-PAHs and the level of DNA adducts and they confirmed that this relation was not linear, a substantial increase in exposure (3–4-fold) was associated with a moderate increase in DNA adduct levels (~20%) [36].

Similarly Moohammadaree and colleagues in 2012 found a significant excess of DNA damage and structural chromosome aberrations in workers who were exposed occupationally to gasoline vapor and air pollutants, when compared to the matched controls [37].

On the contrary, Carere and colleagues in 1995 did not identify a difference in the frequency of SCE among gasoline station attendants and control subjects [30]. Also Bukvic and colleagues in 1998 found no differences in the examined population [38], and finally Trevisan and colleagues in 2014 found the same results in addition that they did not detect the presence of benzene and its derivatives in the blood of exposed workers and did not find chromosomal damage that may be associated with the gas station activity in cases and they attributed that these results might indicate that preventive safety measures of the attendants had been properly done. Also, that owners of gas stations were watched closely by the government and that they provided periodic monitoring of workers' health through medical examinations and laboratory investigations [39].

Regarding hepatic effects, our results shows that, although levels of AST & ALT were within

normal ranges but higher in workers with statistically significant difference between exposed workers and control group ($p < 0.05$) (Table 5 and Graph 1). In exposed workers levels were significantly correlated with the duration of exposure (p value ≤ 0.05). These results should raise our awareness about the early hepatic affection at low levels of exposure that could predict future hepatotoxicity of benzene exposure.



Graph 1. Comparison between exposed and controls in laboratory investigations

Several studies proved this relation through Induction of acute hepatotoxicity in rats by bromobenzene (BB) administration that have resulted in depleted glutathione (GSH) levels and reduced average body weights, 24hr after dosage. BB, a traditional hepato-toxicant, was subjected to cytochrome P450- mediated epoxidation, and a major metabolite is 3,4-epoxide. Detoxication of the active metabolite is by GSH conjugation. At high BB doses, due to the conjugation to the epoxides, liver GSH shortage and secondary reactions such as lipid peroxidation, intracellular calcium alteration, and mitochondrial dysfunction finally led to cell death [40,41]. Also, hepatic centrilobular necrosis was observed at high doses of exposure, the inter individual response varied from very slight to very severe hepatic centrilobular necrosis, a finding which was supported by clinical chemistry parameters [42]. A study done by Pérez and colleagues in 2006 on aromatic hydrocarbons (benzene, toluene, and xylene [BTX]) among workers in a petrochemical company, proved that occupational exposure to aromatic hydrocarbons might cause liver damage proved by rising transaminases levels and ultrasonographic findings [43]. In 2007, Chan and colleagues

proved that the interaction of benzene with cytochrome P450 for oxidation is the metabolic activating path for toxicity [44].

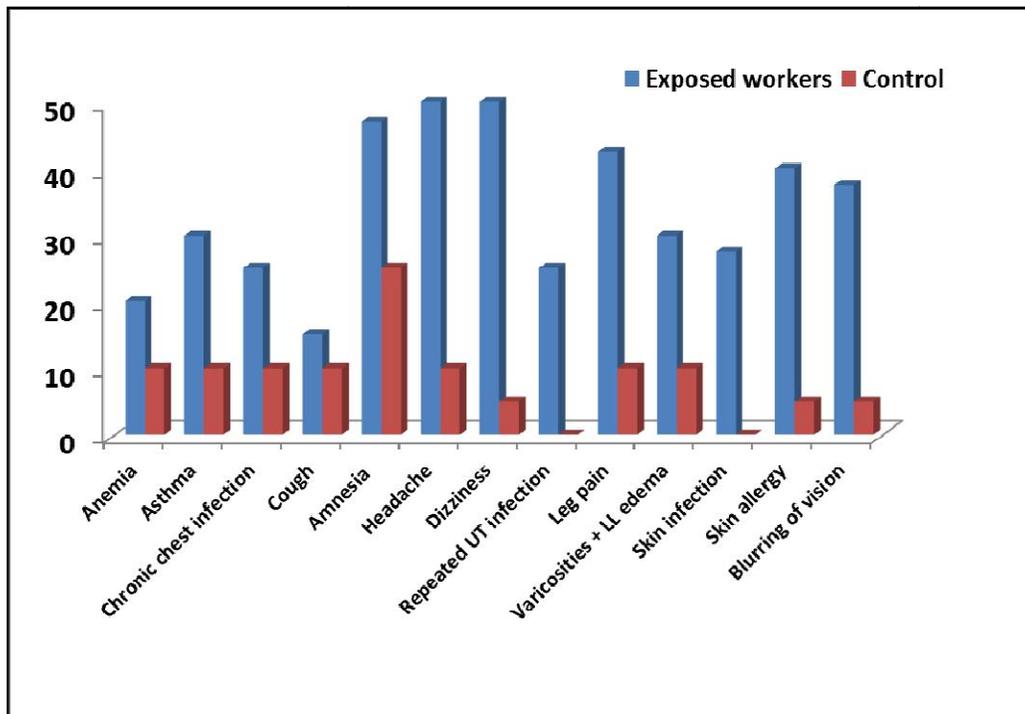
Regarding clinical manifestations, it is assumed the diagnosis of benzene toxicity (or benzenism) when the person exposed presented a specific set of signs and symptoms, characterized by multiorgan involvement, where the blood marrow commitment was the most common and significant component [22]. Signs and symptoms include malaise, myalgia, drowsiness, dizziness, and recurrent infections. The diagnosis of occupational benzenism is essentially clinical, based on the history of occupational exposure and observation of clinical signs and symptoms associated to the laboratory findings [45]. This was consistent with the results of our study which revealed higher incidence of all manifestations among exposed workers with statistically significant difference between exposed workers and control group in (asthma, chronic chest infection, amnesia, headache, dizziness, leg pain, varicosities + lower limb edema, skin allergy, recurrent infections and blurring of vision) (Table 6, Graph 2).

Amnesia and difficult concentration, headache and dizziness were related to chronic central nervous system effects that are consistent with solvent and aromatic hydrocarbon exposures.

Leg pain, varicosities and lower limb edema were related to prolonged standing during work shift. Aromatic hydrocarbons are generally stronger irritants that can cause respiratory tract irritation, skin irritation and dermatitis [46] findings which are present in our study.

Wiwaniitkit in 2005 reported high prevalence of central nervous system symptoms that is consistent with solvent exposure including headache, fatigue, increased sleep requirement and confusion [47].

Similarly, Abou El-Magd and colleagues in 2010, found statistically significant high prevalence of symptoms (drowsiness, dizziness, headache, tremors, sleepiness and confusion) among petrol station workers. They attributed presence of these symptoms to the aberration in basic electrophysiology of the brain and the vasomotor possible mechanism [6].



Graph 2. Comparison between exposed and controls in clinical manifestations

Table 6. Comparison between exposed and control groups in clinical manifestations

	Exposed workers		Control		χ^2	p-value
	Count	%	Count	%		
Anemia	8	20	2	10	3.60	0.058
Asthma	12	30	2	10	7.143	0.008*
Chronic chest infection	10	25	2	10	5.33	0.021*
Cough	6	15	2	10	2.00	0.157
Amnesia	19	47	5	25	8.167	0.004*
Headache	20	50	2	10	1.427	0.000**
Dizziness	20	50	1	5	17.19	0.000**
Repeated UT infection	10	25	0	0		
Leg pain	17	42.5	2	10	11.842	0.001*
Varicosities with LL edema	12	30	2	10	7.143	0.008*
Skin infection	11	27.5	0	0		
Skin allergy	16	40	1	5	13.235	0.000**
Blurring of vision	15	37.5	1	5	12.250	0.000**

All values are represented as mean \pm S.D., *= significant difference at p-value<0.05, **= highly significant difference at p-value<0.001

5. CONCLUSION

The results obtained are valuable, but were only obtained from 3 gas stations in Egypt. It is necessary to better understand the risks that these workers are exposed, so that we can be effective in preventing diseases and maintaining health of these workers.

The demand for gasoline delivery increased; and occupational exposure to mutagenic and carcinogenic agents creates a significant impact on the health status of gasoline station attendants. Bio-monitoring studies on these workers are essential. Further studies are needed to assess DNA status in their lymphocytes that might be a predictor of future cancer risks and might help to prevent further deterioration in the health of these workers.

Identification and early correction or prevention of possible health problems related to such exposure would help in maintaining good health of the workers, prevent reduction in working capacity and loss of working hours beside reducing the cost of medical care for affected workers.

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CONSENT

Informed consent was obtained from each worker before the beginning of the study.

ETHICAL APPROVAL

The study was approved by the local Ethics Committee on human research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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