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Evaluation of the DNA damage and the oxidative stress induced by occupational exposure at gas stations workers

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***Abstract*---**The ambient of Gas stations includes several pollutants, like vehicle exhaust, particulate matter (PM2.5), and violate organic carbon (VOCs) products, occupational exposure to volatile organic carbon (VOCs) are the main components of fuel and vehicle exhaust on the DNA damage, antioxidants, and oxidative stress in addition level of the heavy metals in blood the study carry out in AL-Furat al-awsat consisting of 90 people participated in this study. included 60 workers have been exposed to pollutants of the Gas station and 30 healthy people as a comparison with those exposed. The aim of this study to estimate of the affect of vapor emitted directly from filling stations mainly containing with a lot of saturated and unsaturated hydrocarbon as well as heavy metals (HM) on antioxidants , level of oxidative stress, and DNA damage by shape and size tail length .The results showed decline in antioxidants (SOD), (CAT) at (p-value< 0.0001) and increased in value of (MDA) at (p_value< 0.0001), increased in levels of lead (Pb), cadmium (Cd) with a highly significant at (p_value <0.0001) and cobalt (Co) with a significant at (p_value <0.01) compared with non-exposed, there is significant increase in size of tail length compared with health at (p_value< 0.0001). Conclusion Gas stations workers are more susceptible to pollutants includes VOCs, (HM), and exhaust vehicles lead to the oxidative stress through the (HM) compete with essential trace elements and thus decline in antioxidants also ROS emit from gas stations or formed during metabolites the VOCs in liver, furthermore the DNA damage induce by VOCs mainly and (HM) as secondary because of inhalation of these pollutants for a long period (more than 3 years) and decline in (SOD) and (CAT) in contrast increase in (Pb) , (Cd), and (Co).

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***Keywords*---**gas station workers, heavy metals, DNA damage, comet assay, antioxidants, oxidative stress.

Introductions

Increase demand for energy consumption concurrent with increase in population growth this leads to an increase in the establishment of several fuel filling stations. Volatile organic carbon (VOCs) are major components present in gas stations, VOCs mainly contain aliphatic and aromatic hydrocarbons [1]. Benzene, xylene, toluene, and ethylbenzene are the most common VOCs . Exposure to these compounds occurs through inhalation in addition to vehicle exhaust during refueling . As well as occurs during absorption of these compounds by penetration of the skin [2]. Chronic exposure to aromatic hydrocarbons, including benzene, leads to a series of transformations to cause intermediate compounds and the production of free radicals [3]. The oxidation of benzene take place in the liver by cytochrome 450 to benzene oxide, in addition to reactive intermediates. The enzyme microsomal epoxide hydrolase (mEH) is capable of hydrolyzing benzene oxide to form benzene-dihydrodiol, which is then transformed to catechol or followed by the opening of the trans-transmuconaldehyde ring, which leads to the formation of phenol, which subsequently hydroxylated in the liver to generate hydroquinone (HQ). Fuel filling stations are the sources of many pollutants, including (HM), which are a major component of fuel, including PM10 and PM 2.5 [4,5].

The increase in free radicals, including voliat organic carbon, car exhaust, and the surrounding environmental conditions in those places, and inhaling these radicals leads to oxidative stress, the nuclear factor erythroid-2 (Nrf 2) is responsible for stimulating a series of enzymatic antioxidants and detoxification as an example catalase, and superoxide dismutase, increase in free radicals exposure leads to lipid peroxidation, known as Malondialdehyde (MDA) as well as imbalance between free radical and antioxidants [6,7]. Lead (Pb) is a metal of the most common toxic xenobiotics associated with health conditions disorders. Despite its toxicity, it has been used in a variety of products such as paints, water tape, fuel, and cosmetics due to properties such as low-melting point and corrosion resistance [8,9]. Cadmium (Cd) affects on the mitochondrial compartment by causing changes in intracellular oxidation and reduction states of atoms and lipid peroxidation by inducing oxidative cellular stress, as well as inducing mtDNA mutations [10]. The single cell gel electrophoresis (SCGE) is an effective method for determining DNA repair capacity, resulting from workers exposed to free radical , (VOCs) and metabolism the BTEX refers to the chemicals benzene, toluene, ethylbenzene and xylene [11].

Materials and Method

- The study was conducted in the areas AL-Furat al-awsat for the months from September to November 2021, subjects consisting of 90 people participated in this study. included 60 workers have been exposed to benzene of fuel stations and 30 healthy people as a comparison with those exposed. Hours of the work are 10h at 5 days in week , the mean value for

the exposed workers is (30.5) with range between (19-60 years) and the mean of the control group is [12], with range (19-49 years) , and in comet assay were selected 30 exposed and 30 healthy of the same information in table number (1).

- Questionnaire: All workers exposed during fuel filling stations have at least 3 years of service, and the daily exposure period is 10 hours and 4 days a week. excludes the workers : smoker, have chronic diseases, diagnosed With covid-19 as shown in table (1).

Table 1. Information about the participants

Number phone:		
Age ?		
What is your highest level of education ?		
Weight ?		
Height ?		
Hours of Work ?		
years of Work ?		
Have you smoked in your entire life ?	Yes ( )	No ( )
Do you have a chronic diseases ?	Yes ( )	No ( )
have you previously been diagnosed With covid-19 ?	Yes ( )	No ( )
Do you live in	Urban( )	Rural ( )

- Sample collection: A vein puncture was used to take five milliners of blood from each worker, which was collected in two types of tubes, 2 ml in EDTA tube with store in gold condition such as a wet icebox, 2ml in Gel tube, and the rest in plain tube (no additive)
- Determination of Heavy Metals: Determination of the concentration of (HM) by auto-analyzer (Atomic absorption, by digesting the sample and filtering it through a 0.45 Millipore filter according to [13].
- Determine the SOD, CAT, and MDASOD activity measured through colorimetric methods using Spectrophotometer, according to [14]. MDA using Spectrophotometer according to [34]. Catalase was estimated according to[15].
- Comet assay (DNA damage) measurement the DNA damage according to the shape and size of tail length by [16], and then analysis the picture by comet score V1.5 and image j 1.50i , each sample 100 random cells were selected. The images were capture by the CCD DFC-350-FX Leica and analysis it for 8-bit.
- Statistical Analysis: SPSS software (V.28 Inc., Chicago, USA) and Microsoft Excel 2019 were used to analyse all data. The K-S test is used determination the distribution of variables. Independent t-tests were used to compare normally distributed numerical variables across two groups, with all data represented as mean (SD) Standard deviation.

Results

Heavy Metals

In table (2) shown Difference in concentration value of (Pb) and (Cd) with a highly significant at (p-value <0.0001) increase in workers of gas stations, whereas the (Co) at (p-value <0.01) compared with controls.

Table 2. Shown Difference in concentration value of (Pb) and (Cd)

Heavy materials	Mean ± SD		p-value
	Workers n=60	Control n=30	
Pb (ppm)	0.0585±0.0295	0.0084±0.0012	0.0001 *
Cd (ppm)	0.023±0.0072	0.0043±0.0017	0.0001 *
Co (ppm)	0.0142±0.0042	0.0015±0.0003	0.01

*Significant at p - value <0.05

Table 3. Effects of period of work on (HM) in serum of workers

Variables	Mean ± SD			p-value	LSD
	≤ 3 years	>3-10 years	≥ 10 years		
Pb (ppm)	0.053±0.034	0.069±0.016	0.077±0.014	0.023 *	0.021
Cd (ppm)	0.019±0.001	0.019±0.003	0.022±0.01	0.402	0.007
Co (ppm)	0.013±0.001	0.015±0.003	0.018±0.01	0.199	0.006

Oxidative Stress

The results of various biochemical parameters were compared between gas station workers and a control group in table (3). The results found that workers had a significantly higher serum MDA level than healthy controls at (p-value< 0.0001). Additionally, the statistical found a significant decline in biochemical parameters among gas station workers compared to the healthy group in the following (CAT) , and (SOD) at (p - value< 0.0001) :

Table 4. comparison between healthy and exposed group

Oxidative stress	Mean ± SD		p-value
	Workers n=60	Control n=60	
CAT (µ mol min − 1 mg − 1 protein)	25.23±5.41	32.37±6.75	0.0001 *
SOD Activity (Unit / ml)	0.99±0.32	1.67±0.51	0.0001 *
MDA (µmol/l)	5.05±1.27	1.97±0.52	0.0001 *

*Significant at p - value < 0.05 .

Comet Assay

In table (5) the result of the tail length value in group of workers was greater than health group, whereas the tail length in the workers was greater who has more

years of work, as shown in the table (6) also the comparative of the values for 30 workers with other parameters were chosen the same information for 30 people in study from 60 workers

Table 5. Comparison between healthy and exposed group

	Mean ± SD		
	Workers n=30	Control n=30	p-value
comet assay tail length (µm)	35.88± 2.05	17.19±1.71	0.0001 *

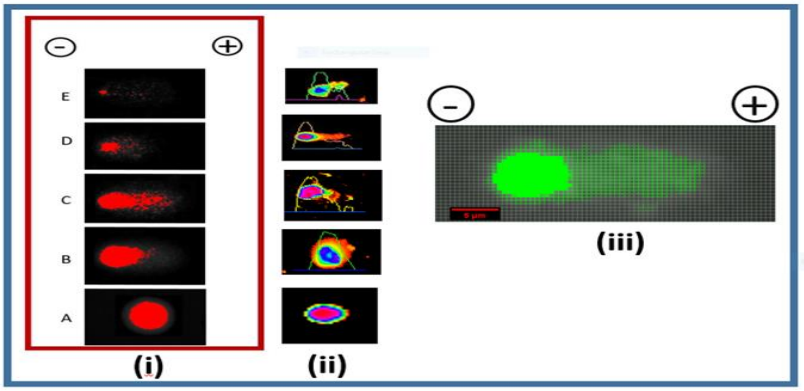

Figure 1. DNA damage according to the size and shape of the tail length

Figure (i) edit by software image j 8-Bit showed the molecules scattered away from the DNA head , moved toward in positive charge in electrophoresis. figure (ii) same sample but in comet score to measure tail length. figure (iii) explain the calculation process by the program when set scale of image.

Table 6. Effects of period of work on comet assay tail length and activity of oxidative in workers

Variables	Mean ± SD			p-value	LSD
	≤ 3 years	>3-10 years	≥ 10 years		
Period of work (year)	3.1±0.23	7±2.11	18.5±4.3	0.0001 *	2.541
comet assay tail length (µm)	34.92±1.74	36.03±2	36.66±2.2	0.162	1.826
CATALASE (µ mol min − 1 mg − 1 protein)	27.64±5.12	26.29±3.93	18.98±1.67	0.0001 *	3.532
SOD Activity (Unit/ml)	1.27±0.24	1.03±0.18	0.57±0.21	0.0001 *	0.193
MDA (µmol/l)	4.22±0.66	5.58±0.73	6.82±0.41	0.0001 *	0.564

Table 7. Effects of period of work on (HM) in serum of workers

Variables	Mean ± SD			p-value	LSD
	≤ 3 years	>3-10 years	≥ 10 years		
Pb (ppm)	0.053±0.034	0.069±0.016	0.077±0.014	0.023 *	0.021
Cd (ppm)	0.019±0.001	0.019±0.003	0.022±0.01	0.402	0.007
Co (ppm)	0.013±0.001	0.015±0.003	0.018±0.01	0.199	0.006

Table 8. Correlation between studied for all biomarkers

		Period of work	comet assay	CAT	SOD	MDA	Pb	Cd
comet assay tail length (µm)	r	0.259	--					
	p-value	0.167						
CAT Activity	r	-0.725**	-0.194	--				
	p-value	0.000	0.304					
SOD Activity	r	-0.807**	-0.337	0.622**	--			
	p-value	0.0001	0.068	0.0001				
MDA Activity	r	0.802**	0.483**	-0.691**	-0.729**	--		
	p-value	0.0001	0.289	0.0001	0.0001			
Pb (ppm)	r	0.426*	0.008	-0.391*	-0.509**	0.474**	--	
	p-value	0.019	0.968	0.033	0.004	0.008		
Cd (ppm)	r	0.164	0.228	-0.062	-0.179	0.106	0.112	--
	p-value	0.386	0.225	0.746	0.344	0.577	0.556	
Co (ppm)	r	0.284	0.245	-0.120	-0.239	0.188	0.175	0.863**
	p-value	0.129	0.192	0.529	0.203	0.319	0.355	0.0001

(*) refer to correlation is significant at p – value < 0.05
(**) refer to correlation is significant at p – value < 0.05

Discussion

Air ambient at workplace (Gas Stations) several chemicals are present such as (HM), whereas workers may inhale and absorb these metals and then settle in body for long time cause potential risk in health. The high concentration of Pb, Cd, and Co results from either through inhalation of air contain these metals or by oral during eating and drinking without washing the hands well before having it. Gas stations are one of the main source of pollution in nowadays, because of toxicity of VOCs, vehicle exhaust, and (HM) take long-period to metabolize and concentration in tissue this for VOCs and accumulation in tissue for (HM), which leading to impact in bone marrow consequently pancytopenia and central nervous as well as DNA damage [17,18].

study have shown that the (HM) can compete with essential trace elements [19], the nephrotoxic impact through the oxidative stress induction (ROS), apoptosis by Ca2+, and mitochondrial disruption. Theoretically Pb^{2+} will competes with calcium and lead to the calcium equilibrium [20,21], (HM) can affect on the epithelial cells in the kidney through the impact permeability and absorption, resulting in kidney disorder and proteinuria level (increase level of protein in urine) [22]. High concentrations of Pb, Ca, and Co in workers, contribute to the increase in the production level of (MDA) , and reducing in the antioxidants (CAT) and (SOD) . The study found significant increase in Pb and Cd, p - value (< 0.0001), while the Co p - value (< 0.01) have been shown increase in concentration with workers have more years of work also there is positive correlation with (MDA) and negative correlation with (CAT) and (SOD).

The increase level of lipid peroxidation in the subjects compared with control, lipid peroxidative damage the cell membrane results from release of free radical OH•- and react with cell membrane which contain polyunsaturated fatty acid lead to destroy the cell membrane and formed malondialdehyde (MDA) [23], in this case the period of years in workers gas stations increase the O2- and H_2O_2 leads to assist of production reactive hydroxyl radical through fenton and haber-wiess reaction follow that OH• react with any parts of components of the cell such as protein , lipids , and DNA [24]. The decline showed in (SOD) activity consider the first line of defense also known the master antioxidant [25] also required cu and Zn [26] , demonstrated the decline of (SOD) back to the competition between essential trace elements (Cu,Zn) and non-essential elements Cd according to [27] , and also Pb compete with Cu because of these (HM) have electron sharing affinities can lead to bind with sulfhydryl groups of proteins [28].

the cobalt enter into the body through inhalation ,and then spread in whole blood , serum , liver , heart , kidney and spleen [29,30], assist to formation of highly reactive oxygen species ROS (straight genotoxic) consequence inhibiting the DNA repair , and damaging biomolecules such as nucleic acid , lipids , and protein according to [31] and also study carry out in city [32] showed accumulation of cobalt also in hair of workers compared with controls in gas stations.

The gel electrophoresis is used to estimate the DNA damage in blood cells. Different morphological changes in nucleus shapes were compared using image analysis. According to single-gel electrophoresis, the nucleus in (A) showed a rounded shape and no deoxyribonucleic acid damage, as shown in figure (3). The nucleus in (B, C, D, E) have a longitudinal egg-shape and was formed of small pieces fractured from the nucleus and scattered longitudinally throughout the picture. The dispersed nucleus in (B, C, D, and E) indicates that the nucleus was migrated to the positive (+) polar region using electrophoresis principles. The image analyses showed that chemical pollutants impacted DNA damage [33]. As a result of deoxyribonucleic acid breakage, the head of the nucleus is shorter, and the tail length is longer transversely [34].

The research investigation there is positive correlation between increase level of (MDA) and size of tail length as well as years of work more than 3 years but there

is no significant correlation between (HM) and tail length . To explain that the increase the tail length in this study come from the VOCs are mainly as shown in statically in table (6) and secondary from (HM) when it compete with essential trace elements, and it leads to exhaustion antioxidants enzymatic or non-enzymatic and thus the cells becomes more vulnerable to free radicals and attack the cell membrane. The head of the nucleus is shortened by adding of OH• to the chain of the C = C bonds of the nucleotides; although, the combination of OH• with (deoxyguansine) is the result of hydrogen atom removal from its exocyclic amine group rather than the adding of hydroxyl radical to the C = C bound. also ROS can stimulate DNA bases modification because of OH• radical attack the guanine [35].

Conclusion

Gas stations workers are more susceptible to pollutants includes VOCs, (HM), and exhaust vehicles lead to the oxidative stress through the heavy metals compete with essential trace elements and thus decline in antioxidants also ROS emit from gas stations or formed during metabolites the VOCs in liver, furthermore the DNA damage induce by VOCs mainly and heavy metals as secondary because of inhalation of these pollutants for a long period (more than 3 years) and decline in (SOD) and (CAT) in contrast increase in (Pb) , (Cd), and (Co).

Ethical approval committee for human research

All study was conducted in accordance with national and institutional guidelines for the protection of human subjects. This study was approved by the Ethics Committee in Research from the Kufa University / faculty of medicine.

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