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Assessment of Sewage Workers' Exposure to Hazardous Materials in Iraq

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ABSTRACT

Background: Sewage workers, who maintain the sewage system and provide an essential service for public health protection, may come in direct contact with sewage hazards, which then may induce DNA damage and oxidative stress. The aim of this study is to assess the level of nitric oxide, homocysteine, 8-OHdG, and vitamins A & E in the plasma of sewage workers for different periods of exposure.

Results: Nitric oxide, vitamin A, and vitamin E showed significant reduction in sewage workers when compared with a non-exposed control group. However, homocysteine and 8-OHdG levels were increased in sewage workers when compared with the non-exposed control group.

Conclusions: Sewage workers are exposed, through different pathways, to a variety of toxicants that cause an increase in homocysteine. Homocysteine, in turn, increased the DNA oxidative damage by increasing the concentration of 8-OHdG and decreasing nitric oxide and vitamins E & A through a decrease in the antioxidant and an increase in oxidative damage.

Key words: sewage hazards, nitric oxide, homocysteine, 8-OHdG, vitamins E & A

INTRODUCTION

Sewage workers, who maintain the sewage system and provide an essential service for public health protection, may come in direct contact with sewage hazards. Sewage water brings contact with many hazardous agents, such as solubilized volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs),[1,2] along with other chemicals, such as heavy metals, fluorinated hydrocarbons, pesticides, nitrosamines, dyes, and polychlorinated biphenyls. [3,4] Many sources for sewage water pollution had been reported as traffic exhausts, industries, waste incinerators, and domestic heating, via both atmospheric deposition and activities of the society. [1,2] Thus, for sewage workers, the sewage workplace environment holds factors that represent a real risk of increased total mortality, of cancer, and of mediating many other health problems.[4,5,6]

Most nitrosamines and nitrite are carcinogenic;[7] although nitric oxide, a free radical, is considered a toxic pollutant in the atmosphere, nitric oxide has a significant physiological role in blood vessels as a vasodilator, enabling pathogen invasion. Peroxynitrite, which is a potent oxidizing agent, results from the reaction of nitric oxide and superoxide. The free radical toxicity of this compound could be attributed to the reaction of peroxynitrite with DNA and proteins. Ordinarily, some immune cells eliminate the nitric oxide's biological effect through the inflammatory response as a part of the body's defense scheme. [8]

Several biomedical studies have found that, at higher concentrations than the physiological level, nitric oxide shows carcinogenic properties and can induce mutation through damage to the DNA. Nitric oxide mediates mutation and DNA damage by its oxidizing derivatives, such as peroxynitrite and other nitrates that can promote DNA damage via single-strand breaks, base deamination, and adduct formation. [9] Homocysteine, which is biosynthesized from methionine by removing the terminal C methyl group, is a highly reactive thiol-containing amino acid. [10] The normal plasma level of homocysteine is between 5 and 15µmol/L. [10] Endothelial cytotoxicity may result from prolonged hyperhomocysteinemia. [12] It has been found that a correlation exists between enhanced homocysteine levels and inflammatory response; varying degrees of hyperhomocysteinemia can be detected with most inflammatory disease. Most of the detected hyperhomocysteinemia has been found not only in patients with autoimmune disease, such as inflammatory bowel disease[13] and rheumatoid arthritis,[14] but also in chronic inflammatory skin disease, such as psoriasis. [15] Moreover, hyperhomocysteinemia has also been associated with other diseases, such as chronic kidney disease,[16] type II diabetes, cardiovascular disease,[17] and cancer.[18,19] Mild hyperhomocysteinemia can frequently be observed asymptomatically in a healthy individual who either is a smoker or who has just been exposed to air pollution. [20] Hyperhomocysteinemia mediates it is effect through stimulation of acute or/and chronic inflammation pathways, such as in endothelial dysfunction, oxidative stress, leukocyte adhesion, and a drop in nitric oxide bioavailability. [21] All these effects have the potential to interact with cellular genetics and result in DNA damage. [22] DNA damage can be detected by measuring several biomarkers. By way of example, 8-oxo-2-deoxyguanosine (8-oxodG) is a marker of guanosine oxidation of nucleic acid, which reveals the level of a DNA lesion. [23] The 8-oxodG may be detected in both blood and urine, although blood assessment of this biomarker represents a part of oxidative stress, while urine assessment of 8-oxodG is potentially considered a noninvasive technique to monitor and assess the overall DNA lesion mediated by oxidative stress. [(24)] An increased 8-oxodG level is an important indicator in the pathogenesis of cancer and other diseases. [(23)]

Limited information is available about the level of 8-oxodG and DNA oxidized bases for sewage workers; however, a significant level of urinary 8-oxodG has been detected in male workers who have been exposed to fly ash at solid-waste incinerators, with specific levels dependent on the duration and level of exposure. [(24)]

This study sheds light on the level of the hazard to which sewage workers are exposed through detection of DNA oxidation mediated by the enhanced concentration of nitric oxide and homocysteine. To this end, different biomarkers, such as 8-oxodG, vitamin E, and vitamin A have been measured in the serum of sewage workers.

SUBJECTS AND METHODS: STUDY POPULATION

In this cross-sectional study, 79 sewage workers were enrolled (60 were males and 19 were females; their ages were between 16 and 65 years of age, with a mean of 37.06±12.18), along with a control group of 40 healthy volunteers with matching age and body mass index (BMI); both groups were from Baghdad. This study was carried out in the Al-Rustumiya plant in Baghdad and the laboratory investigation was conducted in the Department of Chemistry and Biochemistry at the College of Medicine, Al-Nhrain University. All subjects were informed about the purpose, benefits, and risks of the study, as well as their right to withdraw at any time. The study was approved by the Al-Nhrain medical ethics committee.

Subjects and controls with a history of oral contraceptives, vitamin supplementation, or alcohol intake were excluded from the study. None of them had received any topical or systemic medications for at least two weeks before blood collection.

For all workers and controls subjects, the following was done:

Blood specimens were taken between 9:00 and 11:00 in the morning, after 10–12 hours fasting. The blood was collected from a forearm vein into a plain tube and left for 30 minutes to clot before being centrifuged for 15 minutes at 3000 rpm (755xg). The serum was divided into proper aliquots and frozen at -20◦C until used for measuring levels of nitric oxide, homocysteine, and vitamins E and A. All precautions were taken for sample collection and storage in accordance with the Clinical and Laboratory Standards Institute criteria to eliminate metal contamination.

Analysis of homocysteine, vitamins A and E, and β-carotene

High-Performance Liquid Chromatography (HPLC), Shimadzu (Kyoto, Japan) was used. This consists of a system controller, model SCL-10 AVP; a degasser, model DGU-12A; two liquid delivery pumps, model LC-8AVP; a UV-visible detector, model SPD-10AVP; and an injector, model SIL-10A, equipped with a 20 µl sample loop. The HPLC system had been interfaced with a computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacturer. An Epson LQ-300 printer, model P852A (Japan), was used.

Standard preparation

A stock solution of 100 ppm of standard homocysteine (Sigma) was prepared by dissolving 10 mg of homocysteine in methanol and then diluting to 100 ml. The same procedure was followed for vitamins E and A and for β-carotene in the preparation of their stock solutions. Other standard solutions were prepared by subsequent dilution of these stock solutions.

Serum sample preparation

Samples were prepared by adding 50 µl of 15% 5-sulphosalsic to 400 µl of serum, then mixed and centrifuged at 5000 rpm for 10 min. The supernatant was taken and diluted tenfold with distilled water and filtered using minipore filter paper.

Analysis of homocysteine

All samples and standard solutions of homocysteine were chromatographically analyzed with a C-18 column using a mobile phase linear gradient from a 0.1 M acetate buffer (pH4) containing 2% methanol to 0.1 M phosphate, a buffer containing 6% methanol over 15 min (0–100%), a flow rate 0.5 ml/min, and UV-VIS detection at a wavelength of 245 nm.

The normal plasma level of homocysteine is between 5 and 15 µmol/L.[11]. Endothelial cytotoxicity may result from prolonged hyperhomocysteinemia. [12] It has been found that a correlation with most inflammatory diseases can be found with enhanced homocysteine levels, inflammatory response, and varying degrees of hyperhomocysteinemia. Essentially, most of the detected hyperhomocysteinemia has been found not only in patients with autoimmune disease, such as inflammatory bowel disease[13] and rheumatoid arthritis,[14] but also in chronic inflammatory skin diseases, such as psoriasis. [15] Moreover, hyperhomocysteinemia has also been associated with other diseases, such as chronic kidney disease,[16] type-II diabetes, cardiovascular disease,[17] and cancer.[19,20] Mild hyperhomocysteinemia could be frequently observed asymptomatically in healthy individuals who either are smokers or who have just been exposed to air pollution. [20] Hyperhomocysteinemia mediates its effect through stimulation of acute or/and chronic inflammation pathways as, for example, in endothelial dysfunction, oxidative stress, leukocyte adhesion, and dropping of nitric oxide bioavailability. [21] All these effects potentially interact with cellular genetics and result in DNA damage. [22]

DNA damage can be detected by measuring several biomarkers. The 8-oxo-2-deoxyguanosine (8-oxodG) is a marker of guanosine oxidation of the nucleic acid, which reveals the level of a DNA lesion. [(23)] The 8-oxodG may be detected in both blood and urine, although blood assessment of this biomarker represents a part of the oxidative stress; urine assessment of 8-oxodG is potentially considered a non-invasive technique to monitor and assess the overall DNA lesions mediated by oxidative stress. [(22)] An increased 8-oxodG level is an important indicator in the pathogenesis of cancer and other diseases. [(23)]

Limited information is available about the level of 8-oxodG and DNA oxidized bases for sewage workers. However, a significant level of urinary 8-oxodG was detected in the male workers who were exposed to fly ash at solid waste incinerators, depending on the duration and level of exposure. [(24)]

This study sheds light on the level of the hazard to which sewage workers have been exposed through detection of DNA oxidation mediated by an enhanced concentration of nitric oxide and homocysteine. To this end, different biomarkers, such as 8-oxodG and vitamins E and A have been measured in the serum of sewage workers.

Analysis of vitamin A and β-carotene

All samples and standard solutions were chromatographically analyzed with C-18 column using mobile phase methanol–acetonitrile-tetrahydrofuran (75–20-5%), a flow rate of 1.2 ml/min, and UV-VIS detection at a wavelength 290 nm.

Analysis of vitamin E

All samples and standard solutions of vitamin E were chromatographically analyzed with C-18 column using mobile phase ethanol–distilled water (95-5%), a flow rate of 1.0 ml/min, and UV-VIS detection at a wavelength 229 nm.

Analysis of nitrate/nitrite concentrations

Nitrate/nitrite concentrations in plasma were determined using the nitrate/nitrite colorimetric assay kit (Alexis Corp., Nottingham, UK) according to the manufacturer's instructions.

Detection of 8-hydroxyl -2-deoxyguanosine (OHdG) concentration by ELIZA

The 8-hydroxydeoxyguanosine level was measured using an enzyme-linked immunosorbent assay (ELIZA) kit, (BIOTECH-Elx800/England), according to the procedure of the kit, Cayman USA (Item No. 58920).

STATISTICAL ANALYSIS

Values are presented as mean±standard deviation (SD). Statistica version-6 for Windows was used for statistical analysis. Levels of 8-OHdG, homocysteine, nitric oxide, and vitamins A and E were compared in the serum of sewage workers and control subjects. Also, the urine 8-OHdG level was compared by unpaired students' t-tests. The differences were considered to be significant when the p-value was less than 0.05.

RESULTS

The general characteristics of the study population (age, body mass index BMI, and duration of work per year), along with other information for sewage workers and the control group, are summarized in Table 1.

Nitric oxide level in sewage workers

The means of nitric oxide level for the sewage worker group and the non-exposed control group are presented in Table 2. The nitric oxide level showed a significant reduction in all sewage worker groups when compared with the non-exposed control group. Also, among the sewage worker groups, the levels of nitric oxide were significantly different. It has been found that, as the duration of exposure to sewage hazards increased, the nitric oxide concentration decreased.

Homocysteine level in sewage workers

The means of homocysteine level for sewage worker groups and the non-exposed control group are presented in Table 3. The homocysteine level showed a significant increase in all sewage worker groups when compared with the non-exposed control group. Also, among the sewage worker groups, the levels of homocysteine were significantly different. It has been found that, as the duration of exposure to sewage hazards increased, the homocysteine concentration also increased.

8-OHdG level in sewage workers

The means of 8-OHdG levels for sewage worker groups and the non-exposed control group are presented in Table 4. The 8-OHdG level showed a significant increase in all sewage worker groups when compared with the non-exposed control group. Also, among the sewage worker groups, the levels of 8-OHdG were significantly different. It has been found that, as the duration of exposure to sewage hazards increased, the 8-OHdG concentration also increased.

Vitamin A level in sewage workers

The means of vitamin A levels for the sewage worker groups and the non-exposed control group are presented in Table 5. The vitamin A level showed a significant reduction in all sewage workers groups when compared with the non-exposed control group. Among the sewage worker groups, the levels of vitamin A were different but did not reach a significant level. It has been found that the duration of exposure to sewage hazards did not affect vitamin A concentration.

Vitamin E level in sewage workers

The means of vitamin E levels for sewage worker groups and the non-exposed control group are presented in Table 4. Vitamin E levels showed a significant reduction in all sewage workers groups when compared with the non-exposed control group. Also among the sewage worker groups, the levels of vitamin E were significantly different. It has been found that, as the duration of exposure to sewage hazards increased, the vitamin E concentration decreased.

Table 1: Characteristics of the studied population

VARIABLE	CATEGORIES	NON-EXPOSED CONTROL (NEC)	EXPOSED SEWAGE WORKERS (ESW)
AGE	Mean±SD	36.87±11.7 (24–60) Y	37.06±12.18 (1–-65)Y
BMI		25.73±2.2	27.09±4.96
DURATION OF WORK	0–10y 11–20y 21–30y 31–40y		57 (72.2%) 17 (21.5%) 2 (2.5%) 3 (3.8%)
SMOKING STATUS	Smoker Non-smoker		25 (31.6%) 54 (68.4%)
HAEMOGLOBIN HB (MG/DL)	Mean±SD	13.5±1.0	13.9±1.2

Disclaimer	the author has no conflict of interest to declare

Table 2: The means of nitric oxide* (µ mol/L) *value serum of exposed battery manufacturing workers (ESW) according to the exposure time

GROUPS		SAMPLE SIZE	SERUM LEVEL OF NO MEAN±SD (µ MOL/L)
NEC		40	47.5±5.25
ESW	0–10 y	57	32.5±5.5[a***]
	11–20 y	17	25.4±3.5[a***,b***]
	21–30 y	2	20.8±1.2[a***,b,c,]
	31–40 y	3	19.1±0.2[a***,b***,c***,d]

[a] t-test comparison between EMBw exposure time and control***p<0.0001
[b] t-test comparison between exposure time for 0–10 years in manufacturing and other subgroups: ***p<0.0001
[c] t-test comparison between exposure time for 11–20 year period, with 21–30 and 31–40 not significant, ***p<0.0001
[d] t-test comparison between exposure time for 21–30 year period, with 31–40 years not significant

Table 3: The means of homocysteine (ppm) value serum of swage manufacturing workers (ESW) according to the exposure time

GROUPS		SAMPLE SIZE	SERUM LEVEL OF HYS MEAN±SD (PPM)
NEC		40	13.0±1.5
ESW	0–10 y	57	18.3±1.6[a***]
	11–20 y	17	25.3±1.6[a***,b***]
	21–30 y	2	28.1±0.3[a***,b***,c*,]
	31–40 y	3	28.7±0.3[a***,b***,c***,d]

[a]t-test comparison between EMBw exposure time and control***p<0.0001
[b] t-test comparison between exposure time for 0–10 years in manufacturing and other subgroups: ***p<0.0001
[c] t-test comparison between exposure time for 11–20 year period, with 21–30 and 31–40 years not significant, ***p<0.0001
[d] t-test comparison between exposure time for 21–30 year period, with 31–40 years not significant

Table 4: The effect of exposure time on the means of the serum 8-OHdG values of the exposed swage workers (ESW)

GROUPS		SAMPLE SIZE	SERUM LEVEL OF 8-OHDG MEAN±SD (µMOL/L)
NEC		40	100.1±16.33
ESW	0–10 y	57	147.9±19.3[a***]
	11–20 y	17	184.8±19.3[a***,b***]
	21–30 y	2	198.9±0.07[a***,b***,c,]
	31–40 y	5	198.7±0.4[a***,b***c,d]

[a] t-test comparison between EMBw exposure time and control***p<0.0001
[b] t-test comparison between exposure time for 0–10 years in manufacturing and other subgroups: ***p<0.0001
[c] t-test comparison between exposure time for 11–20 years period, with 21–30 and 31–40 years **p<0.001.
[d] t-test comparison between exposure time for 21–30y period, with 31–40 years not significant.

Table 5: The means of vitamin A (ug/dl) value serum of exposed swage workers (ESW) according to the exposure time

GROUPS		*SAMPLE SIZE*	*SERUM LEVEL OF VITAMIN A MEAN±SD (UG/DL)*
NEC		40	59.4±0.97
ESW	***0–10 y***	57	50.86±2.6[a***]
	11–20 y	17	43.5±2.9[a***,b***]
	21–30 y	2	40.5±0.7[a***,b,c,]
	31–40 y	3	40.0±0.00[a***,b***,c,d]

[a]t-test comparison between EMBw exposure time and control***p<0.0001
[b] t-test comparison between exposure time for 0–10 years in manufacturing and other subgroups: ***p<0.0001
[c] t-test comparison between exposure time for 11–20 year period, with 21–30 and 31 –40 years not significant, ***p<0.0001
[d] t-test comparison between exposure time for 21–30 year period, with 31–40 years not significant

Table 6: The means of vitamin E (mg/dl) value serum of exposed swage workers (ESW) according to the exposure time.

GROUPS		SAMPLE SIZE	SERUM LEVEL OF VITAMIN E MEAN±SD (MG/DL)
NEC		40	1.0±0.36
ESW	**0–10 y**	57	0.4±0.1[a***]
	11–20 y	17	0.21±0.06[a***,b***]
	21–30 y	2	0.05±0.05[a***,b***,c***,]
	31–40 y	3	0.1±0.00[a***,b***,c***,d]

[a] t-test comparison between EMBw exposure time and control***p<0.0001
[b] t-test comparison between exposure time for 0–10 years in manufacturing and other subgroups: ***p<0.0001
[c] t-test comparison between exposure time for 11–20 year period, with 21–30 and 31–40 years not significant, ***p<0.0001
[d] t-test comparison between exposure time for 21–30y period, with 31–40 years not significant.

DISCUSSION

Due to their job, sewage workers are exposed to different types of sewage hazards which originate from multiple pathways and cause a complicated matrix of contact with chemicals, having different reactions. [25] Homocysteine displayed a significant increase in sewage workers' serum when compared with that of a control group. Exposure for different constituents of exhaust particles, which are mostly cytotoxic, potentially enhances oxidative stress and increases sulfur-containing amino-acid utilization, such as methionine and cysteine. [26] The present study showed that the homocysteine level was significantly increased in sewage workers (p<0.0001) as the duration of work increased. This was in accordance with Erdogmus et al., who reported that there was a relationship between homocysteine, endothelial impairment, and years of employment. [27]

Excessive production of reactive oxygen species (ROS), which may impair the glutathione-related antioxidant defense system, could be attributed to enhanced plasma homocysteine concentration which, in turn, initiates a further ROS and minimizes antioxidant enzymatic activities. [28] Inflammation pathways of both acute and chronic pathways might be associated with hyperhomocysteinemia, with the consequence of increased oxidative stress and reduced nitric oxide bioavailability. [21] These detrimental effects of hyperhomocysteinemia are attributed to the oxidation of intracellular homocysteine (RSH), which is then released into the blood circulation, not to the oxidation of circulating homocysteine (RS-SR) itself. Intracellular homocysteine (RSH) structurally contains an active sulfhydryl (-SH) group. Once intracellular homocysteine (RSH) is released into the blood circulation, it will react with another homocysteine (RSH) or protein (PSH), creating a disulfide bond (-S-S-); along with this, a superoxide free radical is produced. This process leads to initiating oxidative and nitrosative processes and the production of reactive oxygen and nitrogen species; it also induces inflammation through NF-κB regulation.[29,30] Many reports have revealed a correlation between hyperhomocysteinemia and impairment of endothelium-dependent vasodilatation due to abnormalities in the nitric oxide synthesis process.[31,32] Nitric oxide reveals its important biological role through the inhibition of inflammation, induction of vascular smooth muscle cell proliferation, and promotion of vasodilatation.[33,34] Nitrite and nitrate (indicators of nitric oxide) plasma levels in our exposed group were significantly decreased, as compared with the control group. This result is consistent with the findings of Delfino et al. (2007), who reported that increments of oxidative stress and reduced level of antioxidants and nitric oxide are associated with air traffic pollution. [35] This imbalance in the oxidant/antioxidant system may lead to oxidative DNA damage. However, to the best of our knowledge, this is the first study in which homocysteine, nitric oxide, and DNA oxidative damage expressed as 8-OHdG have been studied in sewage workers in Iraq.

In terms of long periods of exposure to exhaust emissions (especially PM2.5 and SO2, which cause air pollution), it has been found that nitrite and nitrate levels were reduced in traffic policemen as the duration of work increased. These compounds alter nitric oxide bioavailability, not only by the reduction of endothelial nitric oxide synthase activity but also through the acceleration of nitric oxide destruction and generation of reactive oxygen species. [36] Moreover, in hyperhomocysteinemia, auto-oxidation of homocysteine mediates endothelial damage through the promotion of ROS production. [37] Superoxide anion radicals react with and diminish nitric oxide bioavailability with excessive formation of peroxynitrite. [32]

The 8-OHdG is considered to be one the most abundant base modifiers, containing a hydroxyl radical at the C-8 position, which reacts with guanine on DNA.[38,39] The 8-OHdG modifies bases and causes mutation, miscoding results in a variety of abnormalities in protein synthesis. [40, 41]

Our results show there was an increase in the concentration of 8-OHdG in the serum of sewage workers when compared with the control group, and the concentration increased as the duration of the work increased. Andrea et al. stated that both cigarette smoking and air pollution increase plasma homocysteine levels in healthy individuals. Moreover, Luiu et al. noticed that elevated homocysteine may induce lipid peroxidation and DNA hypo-methylation, which may end with cellular DNA damage. [(20)] Once plasma homocysteine concentration increased, reactive oxygen species were increased and the glutathione-related antioxidant defense system was diminished, which lead to reduced antioxidant enzymatic activities and enhanced oxidative stress. [(28, 42)]

Excessive 8-oxodG formation from cellular DNA oxidation due to abundant homocysteine is an important sign, which is frequently initiated in tumor-relevant genes. [(43)] Moreover, deamination and oxidation of bases primarily result in DNA damage, mediated by oxidized products of nitric oxides, such as N2O3 or ONOO–.[(44,45)] In the same way, excessive H2O2 production is highly associated with homocysteine-mediated 8-oxodG formation. 8-oxodG has been considered as a pre-mutagenic in mammalian cells and a putative inducer of the carcinogenic process.[(44,46)] Endothelial cytotoxicity of homocysteine, due to autoxidation of its sulfhydryl group,[(47)] is attributed not only to the reduction of nitric oxide bioavailability but also for both the superoxide and hydrogen peroxide that is generated from oxygen-derived cytotoxic molecules. [(48)] The ROS generated through autoxidation of homocysteine participates in the lipid peroxidation process, which occurs at the surface of endothelial cells and in plasma within low-density lipoprotein particles. [(47)] These ROSs oxidize DNA and affect cell cycle regulation, modifying apoptotic gene expression. [(49)] Friis et al. reported that there is no difference in the level of DNA damage to lymphocytes between sewage workers and construction workers. [(50)] In contrast, the level of DNA damage to the lymphocytes of sewage workers by comet assay found no difference when compared with construction workers, while a significant increase in micronucleus frequency to lymphocytes was described among asphalt workers. [(51)] Moreover, in workers from a petroleum hydrocarbons facility, there was an increase in lymphocyte DNA damage investigated by comet assay. [(52)]

On the other hand, our results show a decrease in the concentration of vitamins E and A. Jordao et al. proposed that the diminished vitamin E levels are probably due to an increased rate of utilization of this vitamin through the detoxification and recovery processes of the animal after ethanol consumption. Jordao and his colleagues show that the level of vitamin E was modified according to the level of ethanol intake through an experiment in which different vitamin E levels (normal, deficient, and supplemented) were used in the presence of acute ethanol intake for the animals, which were then sacrificed at different points in time. The result showed that vitamin E was reduced up to 8 hours after ethanol intake, but that these hepatic levels were restored within a period of 24 hours, except for animals who already had a vitamin E deficiency. [53]

NADPH oxidase is the most crucial enzyme responsible for ROS generation. [54] It has been found that ROSs were decreased by the effect of vitamin E through inhibition of the phosphorylation and translocation of the cytosolic factor p47phox, which results from the reduction of NADPH-oxidase activity. [55]

In conclusion, our data suggest that sewage workers are exposed through different pathways to a variety of toxicants which cause an increase in homocysteine. Homocysteine increases the DNA oxidative damage by increasing the concentration of 8-OHdG and decreasing nitric oxide and vitamins E and A. This decreases antioxidants and increases oxidative damage.

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