



Antioxidants and Some Biochemical Parameters in Workers Exposed to Petroleum Station Pollutants in Mosul City, Iraq

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Abstract

The present study was conducted in Mosul city to evaluate the effects of different pollution sources on antioxidants and some biochemical parameters. The parameters included: Vitamin E, Vitamin A, β -carotene, Vitamin C, folic acid, ceruloplasmin (Cp), total protein, albumin, calcium, total bilirubin, uric acid, creatinine, total iron binding capacity (TIBC), iron, sulfate, glutathione (GSH), malondialdehyde (MDA), cholesterol, glucose, selenium and transferrin saturation(%). The study involved (110) persons divided into two groups, one of which was subjected to petroleum station workers included (51 nonsmokers) worked at different positions and the other considered to be control group (n = 59 nonsmokers)(Outside city center living) with similar age and sex. The results showed a significant decrease in: vitamin E, vitamin C, creatinine, GSH and cholesterol, with no significant changes in: folic acid, calcium, iron, sulfate, glucose, selenium and transferrin saturation (%), vitamin A, β -carotene, total bilirubin and TIBC. On the other hand, there was a significant increase in: Cp., total protein, albumin, total bilirubin, uric acid and MDA. In addition, the increase of workers period in petroleum station cause, a decrease in: vitamin E, vitamin C, folic acid, Cp., total protein, albumin, calcium, GSH, glucose and selenium, and an increase in: vitamin A, β -carotene, total bilirubin, uric acid, creatinine, TIBC, iron, sulfate, MDA, cholesterol, transferrin saturation(%) were observed. It was concluded that an increase exposure of petroleum pollutants to the workers led to an increase in their oxidative stress.

Keywords: Petroleum station, pollutants, workers, antioxidants.

Introduction

Pollution can be defined as the introduction of contaminants into a natural environment, leading to instability, disorder, harm, discomfort and stress to the ecosystem, the physical systems or living organisms. Pollutants included chemicals such as petroleum hydrocarbons, heavy metals, pesticides etc. These contaminants can lead to system stress^{1,2}. Previous studies have demonstrated increased oxidative stress in subjects exposed to environmental air pollution through the assessment of oxidative damage to DNA, lipids, or proteins^{3,4}. For example, free radicals may induce peroxidation of arachidonic acid generating F₂-isoprostanes⁵. Urinary or plasma F₂ isoprostanes have been found to be related to smoking⁶ and exposure to environmental tobacco smoke⁷. Occupational exposure to combustion products, including petroleum have been associated with increased oxidative stress biomarkers in some, but not all, studies^{8,9}. Exposure to petroleum hydrocarbons could be from gasoline fumes at the pumps, spilled oils; chemicals used at home and work e.g. pesticides. Some amount may leak from underground storage tanks and enter the ground waters. Some of the petroleum fractions are released into the soil contaminating the plants grown in such soil^{10,11}.

It was stated under normal physiological condition, animals maintained a balance between generation and neutralization of reactive oxygen species (ROS)¹². However, when organisms are

subjected to petroleum compounds, the rate of production of ROS in cells get increased along with hydrogen peroxide, hypochlorous acid (HClO) and free radicals including hydroxyl radical ($\cdot\text{OH}$) and superoxide anion ($\text{O}_2^{\cdot-}$). Oxidative stress have been implicated in a variety of pathological conditions such as diabetics mellitus, cancer, aging, liver damage, atherosclerosis etc.¹³. Recently, it was reported that airborne particles were associated with decreased in heart rate variability¹⁴. Another recent paper reported nitrogen dioxide (NO_2) and $\text{PM}_{2.5}$ (particulate matter with aerodynamic diameter less than 2.5 μm)¹⁵ were associated with defibrillator discharges due to ventricular arrhythmias in patients with implanted cardioverter defibrillators¹⁶.

Antioxidants such as glutathione (GSH), uric acid, ascorbate and α -tocopherol present in epithelial lining fluid (ELF) may protect the airways from oxidant injury induced by exposure to air pollutants¹⁷. The antioxidants act as sacrificial substrates scavenging oxidant pollutants from the airways and thereby preventing oxidation of macromolecules such as lipids, proteins and carbohydrates.

The aim of the study was to evaluate the effects of pollution for petroleum station workers in Mosul city on antioxidants and some biochemical parameters and to determine the effect of pollution period

Material and Methods

The study included (110) persons represented in two groups. The first group was considered as control (59), while the second group was exposed petroleum pollutants (51) from workers in Mosul city, Iraq.

Kits for determination of total protein no. (0303), albumin no. (0801), calcium no. (2403), total bilirubin no. (0401), iron no. (0502), total iron binding capacity no. (0512), glucose no. (0903), cholesterol no. (0603), were obtained from Syrbio kits, Syria.

The other biochemical parameter (Vitamin A, β -carotene, vitamin E, vitamin C, Folic acid, malondialdehyde, glutathion, selenium, uric acid and creatinine), were estimated using manual methods (table 1).

For venipuncture, 10 ml sterile syringes equipped with (22G x 1.25) syringe needles were used and put of blood in dry and clean plain tubes. After coagulation, it was centrifuged at 4000 x g for 15 minute. Serum was transferred into plain tube equipped with tight-fitting caps by disposable tips, then stored at -20°C^{18} .

Table-1

Methods used to determination of biochemical parameters

No.	Parameters measured	Method used
1	Vitamin A	Needld-Pearson method ¹⁹
2	β -carotene	Needld-Pearson method ¹⁹
3	Vitamin E	Emmerie-Engel reaction ²⁰
4	Vitamin C	2,4-dinitrophenylhydrazine derivatization method ²¹
5	Folic acid	Microbiological assay ²²
6	Glutathion	Modified procedure utilizing Ellman's reagent ²³
7	Malondialdehyde	Thiobarbituric acid method ²⁴
8	Uric acid	Phosphotungstic acid method ²⁵
9	Total bilirubin	Diazo method ²⁶
10	Total protein	Biuret methods ²⁷
11	Albumin	Bromocresol green method(dye binding method) ²⁸
12	Ceruloplasmin	p-Phenylenediamine oxidase method ²⁹
13	Glucose	Glucose oxidase method ³⁰
14	Cholesterol	Cholesterol estrase methods ³¹
15	Creatinine	Jaffé method ³²
16	Total Iron Binding Capacity	Ramsay method ³³
17	Transferrin saturation	Transferrin saturation(%) ³⁴ = Serum Iron /TIBC X 100
18	Iron	Bathophenanthroline method ³⁴
19	Calcium	Methylthymol blue method ³⁵
20	Selenium	Selenium-orthophenylenediamine complex ³⁶

Results and Discussion

Effects of petroleum station on the biochemical parameters of the workers:

The results of different biochemical parameters in petroleum station workers and control group were listed in table (2). There is a significant decrease of biochemical parameters with the workers of petroleum station when compared with the control group in: vitamin E (P=0.008), vitamin C (P=0.0001), creatinine (P=0.0001), glutathione (P=0.0001) and cholesterol (P=0.0001). The decrease is not significant in: folic acid, calcium and iron. This finding agrees with that observation earlier^{37,38}. In addition, the non significant decrease in the levels of sulfate, glucose, selenium and transferrin saturation (%) agree with the results obtained by other³⁷.

Table-2

The biochemical parameters of petroleum station workers and control

Parameter	Control group non-smokers (n=59)		petroleum station non-smokers workers (n=51)	
	mean	SD	mean	SD
Age(year)	35.7	10.9	34.62	7.86
Weight(kg)	76.31	14.5	77.46	10.09
Height(cm.)	169.0	10.57	169.6	8.5
B.M.I(k.g./m ²)	26.71	2.45	26.93	2.45
Vit.E(mg/dl)	1.0	0.21	0.73*	0.18
Vit.A(μ g/dl)	46.42	12.4	58.4	17.7
β -carotene(μ g/dl)	80.49	21.52	162.1	35.22
Vit.C(mg/dl)	0.85	0.17	0.269*	0.02
Folic acid(ng/ml)	6.85	1.7	6.69	1.03
Cp.(mg/l)	151.1	39.4	159.7*	83.1
T.p.(gm/dl)	5.5	0.38	6.25*	0.63
Alb. (gm/dl)	4.36	0.12	4.91*	0.43
Calcium(mg/dl)	11.78	0.24	11.45	0.55
Total Bilir. (mg/dl)	0.41	0.27	0.74*	0.029
Uric acid(mg/dl)	5.05	1.58	5.68*	1.71
Creatinine(mg/dl)	0.82	0.05	0.69*	0.29
TIBC(μ g/dl)	186.8	32.16	219.0	39.69
Iron(mg/l)	1.6	0.19	0.661	0.289
Sulfate(mmol/l)	2.5	1.05	2.47	1.5
GSH(μ mol/l)	13.7	0.89	9.87*	0.86
MDA(μ mol/l)	6.5	2.97	15.06*	3.73
Cholesterol(mg/dl)	180.8	40.13	162.1*	7.94
Glucose(mg/dl)	68.1	35.8	55.21	11.88
Selenium(μ g/dl)	35.7	11.06	34.88	4.46
Transferrin saturation%	36.65	13.5	30.18	10.21

*Different Significantly at $P \leq 0.05$.

Petrol chemical is a complex combination of hydrocarbons. About 95% of compounds in petrol vapors are aliphatic and alicyclic compounds and less than 2% are aromatics. The volatile nature of petrol makes it readily available in the atmosphere at any time it is dispensed, especially at petrol filling stations^{11,39}. A main environmental source of benzene exposure is vehicle exhaust emissions and evaporation losses during the handling,

distribution and storage of petrol⁴⁰. The decrease of antioxidant refers to its utilization which prevents the cellular damage from free radicals, therefore a decrease in vitamin A, vitamin C, vitamin E, glutathion and selenium was observed. Vitamin C is oxidized first as ascorbyl radical and then to dehydroascobate and in the process scavenges free radicals and prevents radical-induced damage of lipoproteins and other macromolecules. Vitamin C was far more effective in inhibiting lipid peroxidation initiated by a peroxy radical initiator than other plasma components, such as urate, bilirubin, and alpha-tocopherol^{41, 42}.

Moreover, it has been observed that there was significantly increase in: ceruloplasmin (P=0.001), total protein (P=0.0001), albumin (P=0.0001), uric acid (P=0.014), Total Bilir. (P=0.029) and malondialdehyde (P=0.02), while there was no significant increase in: vitamin A, β -carotene, total bilirubin and total iron binding capacity. This is in agreement with other studies which illustrated that benzene exposure has been associated with increases in the overall formation of MDA⁴³⁻⁴⁵. It was reported that uric acid might be a consistent and reliable biomarker of significant exposure to lead⁴⁶. The pathophysiology by which lead exposure causes elevation in uric acid level is thought to be due to damage tubules which causes retention of uric acid^{47,48}.

Albumin, uric acid and bilirubin are major antioxidant components of plasma. In addition, albumin might play a major role of the total antioxidant capacity of plasma⁴⁹. Albumin, one of the most important protein in human plasma, is able to bind to Cu^{++} tightly and with iron weakly. Copper bond to albumin is still effective in generating radicals species (Hydroxyl radicals) in the presence of hydrogen peroxide by Fenton reactions. Therefore increasing it to protect human from oxidants compounds formation⁵⁰. Albumin and others, an index or marker of glomerular disease though raised in lead workers were probably a sufficiently reliable of lead nephropathy^{39,40}.

A raised total bilirubin was reported among diesel engine workers who were exposed to high-pressure resistant lubricants containing lead naphthanate⁵¹. They blamed the increase in total bilirubin to the poor compliance of the workers resulting in skin exposure, where lead absorption might have occurred through damaged skin, in addition to that it can be inhaled through the lungs. Many countries have reduced or eliminated the use of lead additives in motor gasoline due to the health consequences of lead exposure as well as to the introduction of catalytic converter, but in other countries leaded gasoline remains the norm, and still acts as a major source of human lead exposure. Although the exposure to lead has declined over the last several decades, lead toxicity remains a public health problem in most industrialized countries^{52,53}.

Effects of working period in petroleum station on the biochemical parameters: Depending on the period workers in petroleum station, the group was subdivided into three groups as listed in table 3.

The results were indicated that vitamin E was decreased significantly with increasing of the period of petroleum station workers while for vitamin C and folic acid non significant difference was observed. Organic components carried on the particle surface play an important role in mediating the toxic effect. For example, polycyclic aromatic hydrocarbons, toluene and benzene can induce oxidative stress indirectly by changing the activities of antioxidant enzymes needed to composite for defense^{54, 55}.

β -Carotene increased significantly with increasing of the period of petroleum station workers, but no significant difference was shown in vitamin A Ceruloplasmin was not affected with increasing the period of petroleum station workers and no significant difference was observed. The total protein wasn't affected, but significant differences in groups was observed, similar results were published³⁹. Albumin was decreased with increasing the period of petroleum station workers and there was a significant difference among the groups.

Calcium was decreased significantly with increasing the period of petroleum station workers and a significant difference was observed among all groups. The increase of subjecting period leads that calcium is readily mobilized in bone deposits^{39,56} causing its level to be decreased.

The total bilirubin was increased with increasing the period of petroleum station workers and significant differences among the groups. This finding was in a good agreement with investigated research^{57, 58}.

Uric acid was increased significantly with the increase of the period of petroleum station workers except last group and a significant difference was observed among all groups. This finding agrees with the other observations found earlier^{40, 59}. It was reported that uric acid is an endoantioxidant, thus raising urate level might in part be an antioxidant responses to protect against the prooxidant effect of lead⁴⁰.

Creatinine was increased significantly with increasing the period of petroleum station workers. The increase level of heavy metals from pollution as the period of pollution increased might increase creatinine⁶⁰. It was found that chronic leads exposure decreases the glomerular filtration rate (GFR) with subsequent renal tubular fibrosis, renal atrophy and disturbances of the renal functions^{61,62}.

Iron and sulfate were increased significantly with increasing the period of petroleum station workers, While TIBC was not affected. This result is in accords with other³⁷.

Glutathion was significantly decreased with the increase of the period of petroleum station workers. GSH is an important antioxidant produced by liver cells and the major soluble antioxidant in cell compartment. The significant decrease in GSH concentration might be attributed to its use in various protective roles against oxidants⁶³.

Table-3

The biochemical parameters of petroleum station workers at different periods of working, and control using Duncan test

Parameter	Control group n=59		(0-5 year) n=22		(6-15 year) n=17		(16year and over) n=12	
	mean	SD	Mean	SD	mean	SD	mean	SD
Age(year)	35.7 a	10.9	31.3 a	10.17	37.66 a	7.58	45.0 a	0.81
Weight(kg)	76.31 a	14.5	74.2 a	11.64	74.33 a	9.33	86.25 a	2.98
Height(cm.)	169.0 a	10.57	167.8 a	6.61	171.0 a	7.53	170.1 a	13.54
B.M.I(k.g./m ²)	26.71 a	2.45	26.35 a	2.31	25.41 a	2.46	29.8 a	1.35
Vit.E(mg/dl)	1.0 a	0.21	0.82 b	0.5	0.68 c	0.27	0.66 d	0.41
Vit.A(μg/dl)	46.42 a	12.4	52.71 a	18.1	63.13 a	19.6	62.18 a	16.28
β-carotene(μg/dl)	80.49 d	21.52	97.7c	45.4	179.7 b	66.1	261.3 a	79.6
Vit.C(mg/dl)	0.85 a	0.17	0.66 b	0.11	0.65 b	0.13	0.57 b	0.03
Folic acid(ng/ml)	6.85 a	1.7	6.8 a	1.64	5.72 a	0.88	5.93 a	89.12
Cp.(mg/l)	151.1 b	39.4	271.9 a	93.7	246.5 a	85.3	219.8 a	77.9
T.p.(gm/dl)	5.5 a	0.38	5.47 b	0.28	5.28 b	0.77	5.89 b	0.5
Alb. (gm/dl)	4.36 a	0.12	4.94 a	1.1	4.05 b	0.39	3.65 b	0.94
Calcium(mg/dl)	11.78 a	0.24	11.64 b	0.3	11.62 c	0.19	10.93 d	0.4
Total Bilir. (mg/dl)	0.41 a	0.27	0.34 b	0.1	0.54 a	0.17	0.46 b	0.29
Uric acid(mg/dl)	5.05 d	1.58	5.62 b	1.9	5.96 a	1.52	5.41 c	1.8
Creatinine(mg/dl)	0.82 d	0.05	0.95 c	0.11	1.21 b	0.07	1.41 a	0.08
TIBC(μg/dl)	186.8 a	32.16	202.8 a	16.2	237.48 a	57.7	208.32 a	13.25
Iron(mg/l)	1.6 a	0.19	0.52 d	0.19	0.69 c	0.34	0.81 b	0.28
Sulfate(mmol/l)	2.5 a	1.05	2.423 a	0.14	2.89 a	0.43	2.69 b	0.83
GSH(μmol/l)	13.7 a	0.89	8.46 b	0.72	7.0 c	0.69	6.31 d	0.56
MDA(μmol/l)	6.5 b	2.97	11.34 b	3.20	15.93 a	4.88	18.41 a	5.01
Cholesterol(mg/dl)	170.8 d	40.13	177.6 c	37.0	182.2 b	10.4	190.26 a	44.04
Glucose(mg/dl)	68.1 a	15.8	58.83 a	14.08	54.71 a	10.0	65.54 a	12.32
Selenium(μg/dl)	35.7 a	11.06	35.42 a	7.21	34.15 b	1.25	32.45 b	5.1
Tansferrin saturation%	36.65 b	13.5	25.64 d	9.21	29.05 c	12.2	38.88 a	13.5

Different letters horizontally a, b, c, d indicate that the means are different significantly at $P \leq 0.05$.

Malondialdehyde was increased with increasing the period of petroleum station workers and a significant difference was observed among each other. Malondialdehyde concentrations was increased significantly, indicating the amount of cellular damage. This increase could be significantly damage, from lipid peroxidation. Lipid peroxidation initiated by free radicals generated as a result of diesel intoxication is usually deleterious to cell membranes and is implicated in a number of pathological conditions⁶⁴.

Cholesterol and transferrin saturation (%) were increased with increasing the period of petroleum station workers, and significant difference among each other. This finding was in a good agreement with others⁵⁹. Moreover an increase in transferrin saturation(%) resulting from pollution was also observed. Aging might increase it with increasing mortality⁶⁵. An increase in serum cholesterol emphasizes liver dysfunction, as cholesterol is synthesized in the liver and also excreted by the liver. The increase in the concentration of LDL-cholesterol is probably because of the increase concentration of cholesterol. LDL-cholesterol could be deposited on the walls of the artery leading to atherosclerosis and other cardiovascular diseases⁶⁶.

High concentration of cholesterol in the serum could lead to diseases like atherosclerosis and stroke⁶⁷. Also high serum cholesterol has been reported to decrease concentration of circulating insulin and serum glutathione⁶⁸.

Glucose was not affected significantly with increasing the period of petroleum station workers and non significant difference among each other in all groups. Selenium was not affected significantly with increasing the period of petroleum station workers.

Conclusion

Conclusion was drawn from the study that, an increase exposure of petroleum pollutants led to an increase in the oxidative stress for station workers. This is due to decrease in the antioxidant levels and an increase in the oxidants especially in the last period of work. This behaviors might suggest that workers subject to petroleum pollutants must supply with antioxidants dose especially with increasing period of exposure.

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