Genotoxic Effects of Lead and Cadmium on DNA of Some Fuel Stations Workers Blood in Hilla City

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Abstract

The study included the genotoxic effects of lead and cadmium on DNA damage of 60 workers of some fuel stations in Hilla city. The results revealed a significant differences (P≤0.05) of lead and cadmium, the concentrations of Pb+2 in blood of control (mean ±SD) was 6.3±0.41 µg/dl while in blood of workers who exposed to fuel for one year, three years and five years were significantly increased and reached (15.5±2.46, 27.4±11.6 and 44.5±17.8) µg/dl respectively. Cadmium concentrations in blood of control was nil while in workers were significantly increased (5±0.72, 12.2±2.6 and 15.3±4.21) µg/dl respectively. Damage criteria was increased significantly (P≤ 0.05) with time of exposure to fuel in comparison with control.

Keywords: Genotoxic; DNA Damage, Heavy metals, Cadmium, Lead.

1. Introduction

Petrol is used worldwide in the industry [1]. The petroleum vapor affects people health by inhalation in addition to accidental ingestion, workers who exposed to petroleum vapor in gas station, drivers of trucks, and workers of refinery are at higher health risk [2]. As reported by the US Agency for Toxic Substances and Disease Registry, petrol composition included more than 150 chemical substances, with small concentrations of toluene, benzene, ethyl benzene, xylene, lead and cadmium [3]. The toxicity of these heavy metals will be depend on their chemical structure, even if including a trace concentrations [4], they will occupy the protein sites in the body of another metals by replacing the original metals resulting in toxicity [5]. Previous studies found that the oxidative damage of bio-macromolecules is as a result of DNA and nuclear proteins with these heavy metals [6].

Lead and cadmium are of worldwide concern, they are found in many environments, so, when workers exposed to them they would be exposed as a co-exposed to other heavy metals because they did not found as a single metal but as a combination with other heavy metals, such as cobalt and nickel [7]. They are considered as a very toxic heavy metals which effect the physiological processes because they does not have any biological role inside the body not like some beneficial metals especially zinc, iron, copper and manganese [8]. The biological monitoring provide us with the information about the form and the route of exposure and measure the doses, the measurement of blood lead is used usually as a parameter to evaluate the exposure of individual to lead while the measurement of cadmium in blood can be used to evaluate the recent exposure to it [9].

Mutagenicity and carcinogenicity of the metals are resulted from the generation of oxidative radicals [10], the DNA repair inhibition [11], glutathione depletion [12] and apoptosis suppression [13]. In addition to that the damaged DNA repair system will accelerate the mutagenesis and genomic instability with different kinds of mutagens [14]. Anyway the molecular-level mechanism which lead to the direct damage of DNA is not clear [15]. The use of suitable biomarker in the assessment is valuable to collect the information correctly [16].

The Comet (single cell gel electrophoresis, SCGE) technique is a rapid, cheap and sensitive technique in order to evaluate the damage of DNA [17]. This assay is of high advantage than other techniques to determine DNA-damage because it is very sensitive [18, 19]. We can observe the degree of damage with this method by evaluating nuclei as tailless or short tail and long tail [20]. The mean concentrations levels of Lead and Cadmium in the petroleum products are given below [21]:
Table 1. Lead and Cadmium concentrations in the petroleum products.

| Sample          | Pb^{2+} | Cd^{2+} |
|-----------------|---------|---------|
| Gasoline (ppm)  | 0.24    | 1.68    |
| Kerosene (ppm)  | 0.41    | 1.33    |
| Diesel (ppm)    | 1.01    | 1.50    |
| standard limits | 0.075   | 5.00    |

2. Methods

2.1. Study area and design

This study was involved the workers of three fuel stations in Hilla city, Babylon Governorate in Iraq, the workers and the control group were not smokers. The workers were divided into three groups according to period of working into fuel station as following; first group was exposed for one year, second group for three years in and third group for five years, in addition to 20 non-exposed persons as control.

2.2. Collection and storage of blood

Samples of blood (5 ml) collected from vein in EDTA tubes, transferred with ice, then stored in refrigerator at 4 °C [22].

2.3. Measurement of Lead and Cadmium

Lead and cadmium extracted by acid digestion method [23]. 10 ml of concentrated HNO₃ was added to 1 ml of blood, treated with heat until being colorless, the solution was completed to 25 ml with de-ionized water after being cool. Then analyzed with a calibrated (Buck 205 flame atomic absorption spectrophotometer).

2.4. DNA damage (Comet Assay)

Blood samples were collected in 0.5 M di-sodium EDTA to avoid formation of clot and 40 µl of blood was taken then, proteinase K (40µl) was added then centrifuged (13000 r.p.m for 15min). Suspension cells (2-5 µl) added to (1.5 ml) tube and mixed with (40µl) of low melting agarose on Comet slide. Lysis solution made (2.5M NaCl, 100 mM EDTA, 10 mMTris-base and 8g NaOH) dissolved and completed to (700 ml) of deionized water, (110 ml) of the mixture (55 ml 1% triton X and 55 ml 10% dimethyl sulphoxide) added, completing volume to (1000 ml) with d.w., chilled at 4°C or by ice for (20 min) at least, spreading the mixture (7.5µl of the previous solution with 75µl of low melting agarose) on a comet slide, warming the slide at (42-50°C) using a heat plate in order to get homogenous agarose and get rid of bubbles. Slides stored with lysis solution (4 C° for 60 minutes), then replacing lysis solution with alkaline solution, pH=13 (6 gm of NaOH and 500 µl of 0.5% Na₂EDTA) for 5-60 min. in dark at room temperature to unwind DNA, slides immersed in 1X TBE buffer for (5 min.) and transferred to a horizontal electrophoresis apparatus, on a flat gel tray and covering the slide with 1X TBE buffer (60 min. volt 70), tapping off the excess TBE gently by adding many drops of (70% ethanol) later, staining the slides with ethidium bromide and viewed by fluorescence microscope after 24 hour [24-25].

2.5. Statistical Analysis

Data analyzed with (SPSS 10.00). All data were recorded as (mean ± standard deviation (SD)) applying t test (P≤0.05).

3. Results

3.1. Lead and Cadmium in blood

Results of present study were showed a significant differences of lead and cadmium between control and workers at (P≤0.05), the lead concentration in control (mean ±SD) was 6.3±0.41 µg/dl while its in group 1 (workers who exposed for one year) was significantly increased and reached 15.5±2.46 µg/dl, group 2 (workers who exposed for three years) was elevated to 27.4±11.6 µg/l and in group 3 (workers who exposed for five years) was 44.5±17.8 µg/dl figure (1 a). The cadmium concentration in blood of control was nil, while its concentration in blood of workers who exposed for one year was reached 5±0.72 µg/dl, in workers who exposed for three years 12.2±2.6 µg/dl and in workers who exposed for five years was 15.3±4.21 µg/dl figure (1 b).
Figure 1. (a): The concentrations of lead in blood of group 1, group 2 and group 3, (b) The concentrations of cadmium in blood of group 1, group 2 and group 3

3.2. DNA Damage

According to figure, the DNA damage could be classified into three classes of damage; first class: low damage, second class: medium damage and third class: high damage (figure 2). DNA damage criteria increased significantly at \((P\leq 0.05)\) with increasing period of exposure to fuel vapor as compared with control.

Figure 2. Classes of DNA damage in blood according to comet assays, (a) Low Damage (b) Medium Damage (c) High Damage (40X).

3.3. Comet length

In one year exposure (group 1) the comet length was 109.14 \(\mu\)m, while in three years exposure (group 2) was reached 137.77 \(\mu\)m and in five years exposure (group 3) reached 145.32 \(\mu\)m in comparison with the control 51.90 \(\mu\)m (figure 3).

Figure 3. Comet Length (\(\mu\)m) in blood.

3.4. Tail length

Tail length value in control was reached 37.95 \(\mu\)m, while tail length in workers of fuel station was (100.57, 115.8 and 134.09) \(\mu\)m for one, three and five years of exposure respectively (figure 4).
3.5. Tail DNA %

Tail DNA % in control was reached 22.91%, while in workers reached (94.96, 99.93 and 99.85)% for one, three and five years of exposure respectively (figure 5).

3.6. Tail moment

In one year exposure group the tail moment was 100.5 µm, in three years group reached 97.29 µm and in five years group reached 133.99 µm in comparison with the control 36.75 µm (figure 6).

4. Discussion

Fuel exposure danger resulted from mutagenic and carcinogenic effects of the main components [26]. The results of present study revealed a significant differences at (P ≤ 0.05) of lead and cadmium in comparison with control, the concentrations of one year exposure was significantly increased and reached 15.5±2.46 µg/dl, in workers who exposed for three years the concentrations elevated to 27.4±11.6 µg/l and in workers who exposed for five years the concentrations was 44.5±17.8 µg/dl. The cadmium concentrations in blood of control was nil, while its concentrations in blood of workers who exposed for one year reached 5±0.72 µg/dl, in workers who exposed for three years reached 12.2±2.6 µg/dl and in workers who exposed for five years was 15.3±4.21 µg/dl.

Moderate and long term exposures to fuel causing accumulation of heavy metals in addition to an increase of oxidative stress as a result of non-use of facial masks during work which increases the level of heavy metals in blood due to inhalation of them with polluted air. Didn't use of protective clothing and body wash after work may also increase the dermal absorption [27, 28].
The results of DNA damage (comet and tail length, tail DNA% and tail moment), all these markers of DNA damage increased significantly (P≤ 0.05) in comparison with control due to accumulation of metals which led to formation of oxidative stress via generation of free radicals (superoxide, hydroxyl radicals and Reactive Oxygen Species (ROS)) which decreasing the antioxidant activity, protein denaturation, lipid peroxidation and DNA damage which causing numerous conditions detrimental to health [29, 30].

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