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Research Article

Plasma Concentration of Cadmium, Lead and Chromium in Smokers and Nonsmokers in Tripoli, Libya: A Comparative Study

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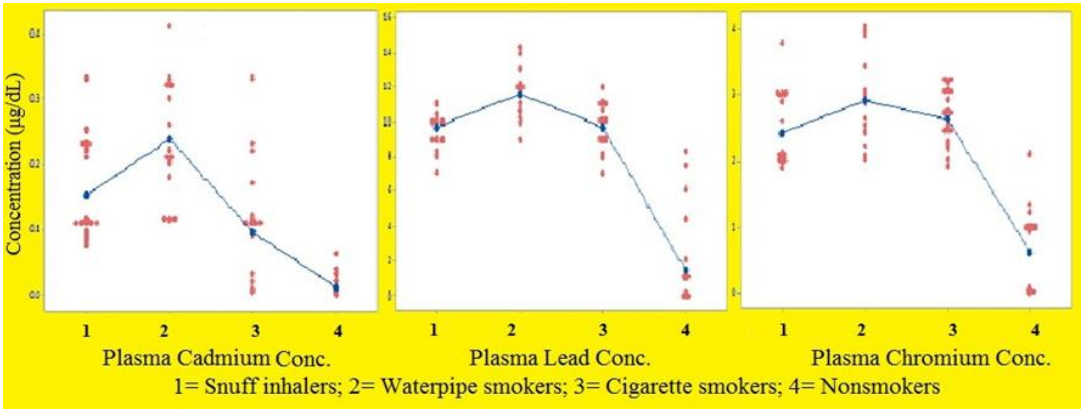
ABSTRACT

Tobacco products are known to contain some heavy metals that pose threats to smokers. In order to assess the role of smoking habits in increasing plasma concentration of cadmium (Cd), chromium (Cr) and lead (Pb), a comparative study between male smokers and nonsmokers was carried out in Tripoli, Libya. Graphite furnace atomic absorption spectrometry (GFAAS) was used to determine the concentration of Cd, Cr and Pb in plasma blood of 25 male nonsmokers representing control group, and of 75 male smokers divided equally into three subgroups, which were: cigarette smokers group, water pipe group and snuff inhalers group. According to 2-sample test, the means (or the medians) of Cd, Cr and Pb plasma concentrations were significantly higher in smokers groups compared to the nonsmokers group, and as the one-way analysis of variance (ANOVA) test revealed, the means of Cd, Cr and Pb plasma concentrations were significantly higher in water pipe group compared to the other two smokers' groups. In conclusion, smoking increased the plasma concentration of Cd, Cr and Pb, and smoking water pipe is considered to elevate the plasma concentration of those three metals more than the other two smoking habits, thus it is more dangerous on smokers' live.

GRAPHICAL ABSTRACT



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Introduction

Nicotine addicts in Libya get their daily dose of this toxicant by different smoking habits including smoking cigarette, smoking water pipe (narghile, hookah, shisha) and inhaling snuff, known locally as naffa. In addition to nicotine, it has been reported that tobacco products contain other toxic substances including some heavy metals [1-3]. It is believed that smoking contributes in increasing the blood concentration of some heavy metals such as cadmium (Cd) [4,5], lead (Pb) [4] and chromium (Cr) [6]. Many heavy metals are known to have adverse impact on human's health, even at relatively low-level exposures [7], and are suspected of causing cancer and many other life-threatening diseases such as cardiovascular diseases [8].

For Cd, The major exposure sources in humans are food consumption and smoking [7], and since lungs absorb cadmium better than the gastrointestinal tract [9], it is expected that there is higher exposure to cadmium from smoking rather from food consumption. Through smoking, cadmium enters the lungs then builds up in tissues, particularly the liver and kidneys [10]. It is excreted in urine at slow rate [5]. It is also reported that the long-term accumulation of cadmium can adversely impact health in many ways including: cancer [11], kidney damage [12] and bone demineralization [13].

In case of Pb, human exposure occurs through ingestion or inhalation [14], it accumulates in bones and teeth [15] while excreted through the feces and also in the urine [16]. The half-life of lead, which is stored in bones, could be up to nearly 48 years, reflecting that it has a long-term effects on health [17]. Pb is considered as a carcinogen [18] and numerous studies have pointed out it is associated with hypertension [19], chronic kidney disease [20] cognitive and neuromuscular decline [21].

As for Cr, the main routes for human exposure are inhalation, ingestion and skin contact [22]. It accumulates in bones, spleen, liver and kidneys [23] and excreted mainly in urine [24]. Among the most common forms of Cr is Cr^{+6}, to which the toxicity of this metal is mainly attributed [25]. This form is classified as a carcinogen by the International Agency for Research on Cancer [26] and by the World Health Organization [27]. Cr^{+6} is also known to induce DNA damage [28] and to cause hypersensitivity reactions such as asthma [29], kidney damage such as acute tubular necrosis [30] and dermatitis [25].

Previous studies have reported that cigarettes [2,31], water pipe tobacco [32] and snuff [31] contain Cd, Pb and Cr, with amounts that vary depending on the brand. Therefore, it is expected that smoking habits have their role in increasing the concentration of the above mentioned metals in smokers' blood, consequently endangering their lives.

Thus, the aim of this study was to compare the concentrations of Cd, Cr and Pb in plasma blood of male smokers (cigarette smokers, water pipe smokers and snuff inhalers) with their concentrations in plasma blood of male nonsmokers as a control group. Moreover, the plasma concentration of Cd, Pb and Cr in the three smokers group were compared to each other.

To achieve this, the concentration of these three metals in plasma blood was measured for all subjects by graphite furnace-atomic absorption spectrometry (GFAAS) then the obtained data were compared and treated statistically. This approach would show to what extent these three smoking habits contribute in increasing the concentrations of Cd, Pb and Cr in smokers' plasma blood and to evaluate how dangerous they are to smokers' lives.

Experimental

Subjects

The total number of subjects was 100

Libyan males who were resident of Tripoli, Libya. Twenty five of them were nonsmokers and 75 were smokers. Male nonsmokers were from the Military Academy of Marine Studies, Janzour, Tripoli, Libya. Male smokers group consisted of three equal-in-number subgroups, those were: cigarette smokers smoking, water pipe smokers and snuff inhalers, with 25 subjects for each subgroup. The subjects of each group were selected on the condition that they smoke (or inhale) the same brand. Age range for the nonsmoker group and each smokers' group was 25-45 years, with a mean of 35 years for nonsmokers, 36 years for cigarette smokers and 34 years for both of water pipe smokers and snuff inhalers.

Blood sampling

Blood sampling was carried out at Salah Aldeen Hospital (Tripoli, Libya) after acquiring the permission form the head of the hospital. Blood samples (4-5 mL) were withdrawn from a cubital vein of each participant, transferred into a test tube with anticoagulation agent (lithium heparin tube) and stored at 4 °C, then transported to the Biotechnology Research Center, Tripoli, Libya.

Measurements of metals concentration

Plasma separation and analysis for its content of Cd, Pb and Cr were performed at the Biotechnology Research Center, Tripoli, Libya as described in literature [33]. All reagents used were of analytical grade and purchased from Sigma-Aldrich, St. Louis, MO, USA. To prevent any contamination all the used glassware and polyethylene containers were soaked in 5% nitric acid for 24h, cleaned with deionized water, and dried. All samples were analyzed for concentrations of plasma Cd (P-Cd), plasma Pb (P-Pb) and plasma Cr (P-Cr) using GFAAS, (Shimadzu, AA-6800) The wavelengths for Cd, Pb and Cr were set to 228.8, 283.3 and 357.9 nm, respectively, and the spectral bandpass was set to 0.7 nm.

Statistical analysis

All statistical analyses and graph drawings were performed using Minitab 17 (Minitab Inc., State College, Pennsylvania, US). Descriptive statistics including mean, minimum and maximum values were carried out for each group in order to run a comparison between the groups. T-test (for means) and Mann-Whitney test (for medians) were applied in order to investigate the significance of difference (α=0.05) between each smokers' group and the control group regarding P-Cd, P-Pb and P-Cr concentrations. T-test was applied whenever each pair follow the normal distribution, otherwise Mann-Whitney test was applied. One-way analysis of variance (ANOVA) test was employed to investigate if there were significant differences (α=0.05) between the means of P-Cd, P-Pb and P-Cr concentrations of the three smokers groups, and thus to conclude which smoking habit contributed more in increasing these concentrations.

Results and discussion

All concentrations of P-Cd, P-Pb and P-Cr in the three smokers' subgroups (except for 7 readings of P-Cd in cigarette smoking group) were higher than the means of P-Cd, P-Pb and P-Cr concentrations in the control group as shown in Figures 1-3.

Table 1 shows that the mean, the minimum and maximum values of the three metals in plasma of smokers were higher than those of control group. According to the results of t-test, the means (or the medians) of P-Cd, P-Pb and P-Cr concentrations in the three smoking group were found to be significantly higher compared to nonsmokers group ($p<0.05$). Similar results have been reported in previous studies in which the concentrations of these metals have been measured in sera of smokers and nonsmokers [4, 34].

The highest mean values for P-Pb, P-Cd and P-Cr concentrations were recorded in water pipe smokers' subgroup, suggesting that smoking water pipe led to increasing the plasma concentration of these three metals more than smoking cigarettes and inhaling snuff. This could be attributed to many reasons including the high concentration of these heavy metals in water pipe smoke. It has been reported that most toxic metals, such as cadmium, lead and chromium, present at much higher concentrations in water pipe smoke compared with cigarette smoke [35].

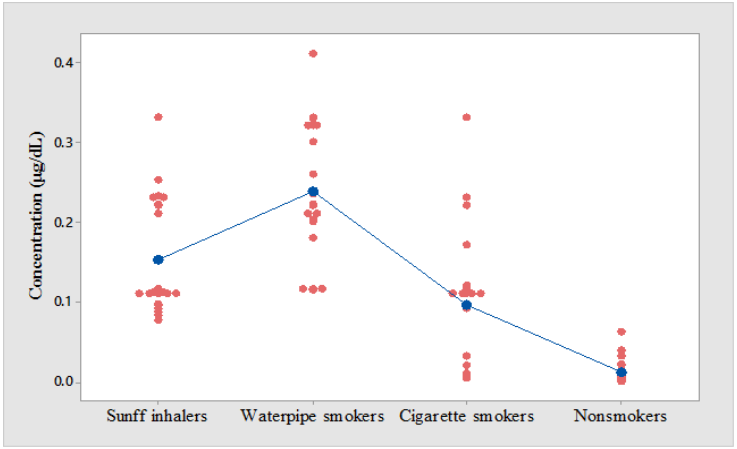

Figure 1. Individual value plot of P-Cd concentrations in all groups with a line connecting the means

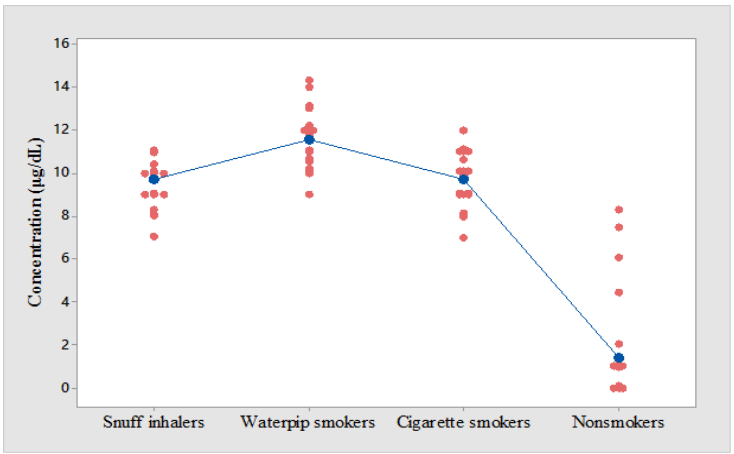

Figure 2. Individual value plot of P-Pb concentrations in all groups with a line connecting the means

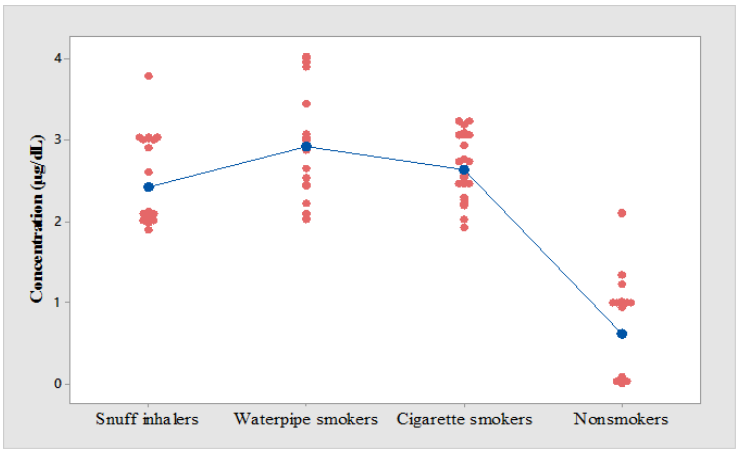

Figure 3. Individual value plot of P-Cr concentrations in all groups with a line connecting the means

Table 1. Shows the mean, minimum and maximum concentrations of P-Cd, P-Pb and P-Cr (µg/dL) in all subjects included in the study

Metal	Group	Mean	Minimum	Maximum
P-Pb	snuff inhalers	9.705	7.076	11.088
	waterpipe smokers	11.56	9.011	14.342
	Cigarette smokers	9.712	7.032	12.012
	nonsmokers	1.412	0.004	8.321
P-Cd	snuff inhalers	0.153	0.078	0.332
	waterpipe smokers	0.239	0.116	0.412
	Cigarette smokers	0.097	0.006	0.332
	nonsmokers	0.012	0.001	0.064
P-Cr	snuff inhalers	2.425	1.908	3.785
	waterpipe smokers	2.917	2.022	4.032
	Cigarette smokers	2.640	1.933	3.233
	nonsmokers	0.619	0.012	2.111

Other reasons are: the burning temperature of tobacco [36] puffing frequency, inhalation depth, length of smoking sessions [37] and the variance of these three metals amounts in the brands. The relatively higher increase of P-Cd, P-Pb and P-Cr concentrations in water pipe group indicates that smoking water pipe is more dangerous to smokers' lives than the other two habits of smoking when considering these three metals.

Elevation of P-Cd, P-Pb and P-Cr concentrations in the three smokers' group could leads to many health disorders including cancer. Increase of P-Cd concentration could lead to dysfunction of the kidney, osteoporosis [38], while increase of P-Pb concentration could lead to anemia, increase in blood pressure and kidney damage [39] and the increase of P-Cr concentration could lead to leukocytosis or leukopenia and decrease in hemoglobin level [40].

The results of one-way ANOVA test revealed that there was significant difference ($p<0.05$) between the means of P-Pb concentrations of the three smokers' subgroups, and that P-Pb concentration values in water pipe group (with the highest mean) has a significantly higher mean compared to cigarette smokers and snuff inhaling groups. Thus, water pipe smoking habit contributed in increasing concentration of P-Pb more than the other two smoking habits. In addition to that, the means of P-Pb concentration of cigarette smokers and snuff inhaling groups were not found to differ significantly from each other, suggesting that both of these smoking habits contributed in increasing the P-Pb concentration to the same extent.

In case of Cd, the results of ANOVA test showed that there was significant difference ($p<0.05$) between the means of P-Cd concentrations of all the three groups, suggesting that each smoking habit increased the P-Cd concentration to different extent. The difference was more significant when the P-Cd concentration of water pipe group (with the highest mean) was compared with P-Cd concentrations of cigarette smoking group

(with the lowest mean). The results suggest that water pipe smoking contributed in increasing P-Cd concentrations more than the other two smoking habits and the order of increasing P-Cd concentrations was as follows: water pipe smoking>snuff inhaling>cigarette smoking.

As for Cr, ANOVA results pointed out that the most significant difference ($p<0.05$) was between the means of P-Cr concentrations of water pipe smoking group (with the highest mean) and snuff group (with the lowest mean), suggesting that smoking water pipe increased the P-Cr concentration more than inhaling snuff. Although the mean of P-Cr of cigarette smoking is smaller than that of smoking water pipe group and higher than that of inhaling snuff group, it was not possible to conclude that it contributes in increasing P-Cr concentration to different extent because it was not found to differ significantly from the means of the other two groups.

Conclusion

The three smoking habits (water pipe smoking, cigarette smoking and snuff inhaling) contributed in increasing the P-Cd, P-Pb and P-Cr concentrations significantly in the studied smokers. Regarding the P-Cd, P-Pb and P-Cr concentrations, the water pipe smoking habit poses a more dangerous threat to smoker's life compared to cigarette smoking and snuff inhaling habits. Other similar studies are needed to investigate the role of smoking habits on increasing other heavy metals such as nickel, cobalt and arsenic.

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