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Simultaneous Detection of Toxic and Heavy Metals in the Scalp Hair Samples of Smokers

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Abstract

Toxic and heavy metals are non-biodegradation pollutants in the environment that can affect human health. The levels of these metals can be analyzed with some biological materials, and they may be used to monitor changes in the human body. The levels of selected toxic and heavy metals (Hg, Pb, Ni, Se, Mn, As, Cr, Cd, and Zn) in the scalp hair of male smokers and non-smokers were determined to investigate dietary exposure to various chemical metals pollutants in Saudi Arabia Population. All investigated samples were collected from cigarette smokers, hookah smokers, and non-smoker (control) volunteers in Hail city. The samples were digested using microwave acid digestion, after which the analysis was carried out by inductively coupled plasma mass spectrometry (ICPMS). The data obtained from control samples were used for purpose of comparison and the smoking state of the family was considered. A systematic analysis investigation was carried out for all samples taking into consideration the number of smoked cigarettes and/ or hookah per day as well as the smoking duration. Finally, the obtained results showed the presence of a statistically significant relationship between smoking and the heavy metal concentration detected in smoking samples, which may play a very important guide for many respective antismoking organizations.

Keywords: Heavy/toxic metals, Smokers, Scalp hair, Microwave acid digestion, ICP-MS, Saudi Arabia

Introduction

Environmental pollution is a remarkable factor in various chronic diseases of human health due to the highest levels expose of toxic and heavy metals that have various sources, such as natural gas and petroleum, burning of coal, tobacco, and incineration of hazardous waste worldwide (Momčilović *et al*., 2016; Cabar *et al*., 2020; Almeida-da-Silva *et al*., 2021; Yin *et al*., 2021). In this context, many carcinogenic materials, including heavy and toxic elements represent a major factor in various chronic diseases such as cancer, lung and cardiovascular diseases, as well as genetic defects. So, perfect determination of these metals in the human tissues is very important. Human life or histories such as habit, food, sex, age, and social condition leads to the presence of personal differences in the concentration of these metals in the human body (Momčilović *et al*., 2016; Ikese *et al*., 2021). On the other hand, analysis of certain biological materials including blood, teeth, nails, and hair; is possible to monitor changes in the body. Smoking has come to be one of the important sources of exposure to heavy and toxic metals in different organs of the human body (Alrobaian & Arida, 2019). Therefore, accurate determination of the concentration of these metals in human fluids and/or tissues is highly important to investigate the effects of smoking. Different analytical tools have been considered for the measurement of heavy and toxic metals in various biological samples (David *et al*., 2016; Mehra & Thakur, 2016; Silva *et al*., 2018; Ocelić Bulatović *et al*., 2019; Noreen *et al*., 2020; Al-Muzafar *et al*., 2021; Lotah *et al*., 2021). The biomaterials structure of hair has a long-term accumulation with many trace elements that can provide data for determining the health status of the human body (Nakaona *et al*., 2020). For this reason, hair samples can be used as great valuable tissue as a the monitor for exposure of individuals to various chemical pollutants, including the effects of smoking. On the other hand, toxic and heavy metals enter the human body through various sources; including smoking can cause physiological disorders (Ullah *et al*., 2017; Rajfur *et al*., 2018). In this context, the trace toxic and heavy elements (Pb, Cd, Se, As, Ag, Al, Ba, Cr, Sr, V, Ni, and Hg) should not be present in the human body, it causes increased sensitivity to various diseases such as cancer, diabetes, mutagenicity and cardiovascular diseases (David *et al*., 2016; Mehra & Thakur, 2016; Ullah *et al*., 2017). The minor elements present in lower levels, for example, Fe, Mn, Zn, Cu, and Co are also essential as metabolic agents as well as enzymes catalysts. While, the major metals present in high levels such as Mg, Ca, Na, and K are critical to the metabolism as well as the activity of the human body, too much of one mineral may become toxic (Ullah *et al*., 2017; Shin *et al*., 2020). On the other hand, toxic and heavy elements are very dangerous can cause many illnesses such as lower energy levels, damage to lungs, kidneys, liver, and other vital organs, chronic or acute poisoning even present in trace levels.

Many researchers (Liang *et al*., 2017; Pozebon *et al*., 2017; Watanabe *et al*., 2019; Polak-Witka *et al*., 2020) observed that scalp hair has not only the rare ability to disclose the heavy metals introduced into the human body, but is also useful as a measure of environmental contamination as well as disclose historical details on the dietary state and chronic diseases. In addition, they found that certain foreign metals are present in hair samples at a rate more than 10 times at least in urine or blood serum samples. In other

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words, scalp hair samples have come to be one of the most important indexes in such studies.

Baker and Ward (2018), reported that hair samples have many advantages over other biological samples such as easy storage, metals accumulate over a long time without any changes, a long-term growth substance, and many trace metals present over two to eighteen months. They also found a relationship between these elements and human health. Recently, many studies have been reported to estimate the relation between the presence of toxic and heavy metals in human tissues samples, and the smoking habits, to explain whether smoking is the origin of these hazardous metals using different analytical tools such as atomic absorption spectrometry (Alrobaian & Arida, 2019; Wu *et al*., 2021).

For this reason, hair has come to play an important role in this subject as worthy tissue for the investigation to monitor the exposure of individuals to environmental pollution. In this context, the use of mineral investigation of hair was also accepted by the International Atomic Energy Agency (IAEA) to estimate the levels of toxic and heavy metals in living organisms, including humans (Gönener *et al*., 2020). Recently, inductive coupled plasma mass spectrometry (ICP-MS) as modern analytical techniques is helpful for the determination of toxic and heavy metals at part per billion (ppb) in human tissues samples due to the following advantages; small sample quantities needed (3-5 ml for solution), high sensitivity, high precision, and accuracy, rapid quantitative and semi-quantitative analysis of trace elements, multi-element technique (Pozebon *et al*., 2017; Gönener *et al*., 2020). The present study aimed to determine the level of toxic and heavy elements (Pb, As, Se, Hg, Mn, Cd, Cr, and Ni) in scalp hair of smokers and non-smokers in the Saudi Arabia population using ICP-MS. The goal of this study is to determine the level of toxic and heavy elements (Pb, As, Se, Hg, Mn, Cd, Cr, and Ni) in scalp hair of smokers and non-smokers in Hail city, Saudi Arabia by using ICP-MS as modern analytical techniques. On the other hand, the present study aimed also to explain the relationship between smoking, as well as the smoking period, and the presence of toxic and heavy metals in the hair.

Materials and Methods

All reagents used in this work are AR grade and there is no need for further purification. Deionized water with 18 MΩ cm^{-1} was used to prepare all standard solutions, dilute samples, and wash all tools and glassware. Hydrogen peroxide (H_2O_2, 35%), and nitric acid (HNO_3, 69%) were obtained from Sigma-Aldrich. All plastic and glassware used were washed and cleaned with dilute nitric acid, and air-dried before use. In all the digestion processes, a microwave digestion system (Anton Paar -Multiwave PRO, USA) was used. The reaction vessels were rinsed with about 7 mL of concentrated HNO_3 and then washed with deionized water, while the hair samples were washed with a mixture of triton X-100 and acetone, before each digestion. In all digestion steps, Teflon containers were used with the investigated hair samples.

After the drying process, each investigated sample (0.5 g of hair placed in a Teflon container) was individually digested in the microwave digestion system with a mixture of 7mL of concentrated HNO_3 and 3 mL of H_2O_2. In this system, the temperature ramped to 200°C for 10minutes, after which held for10 minutes, and then cooled to reach room temperature. The digestion method used includes several advantages such as; minimum sample handling steps which lead to minimizing the sample contamination, and the large number of samples, which lead to improving the overall reliability of the suggested analysis protocol. The microwave procedure operated according to the report by the US Environmental Protection Organization (USEPA) (Schweitzer & Cornett *et al*., 2008). After cooling of the digestion vessel, the resulting clear solution was quantitatively diluted with deionized water and an aliquot was inserted into the cell for analysis using ICP-MS. A blank sample, containing deionized water was also used under the same conditions.

The investigated toxic and heavy metals were measured by inductively coupled plasma mass spectrometer- ICPMS2030 (Shimadzu- Japan) under optimized plasma conditions in all digested samples and blanks as well. The concentration of the investigated metals was measured automatically using the standard graph, which was prepared under the same plasma conditions. The wavelengths (nm) used in the detection of Pb, As, Se, Hg, Mn, Cd, Cr, and Ni were 220.353, 189.042, 196.090, 184.950, 257.610, 228.802, 283.563, and 221.647, respectively. The system was adjusted to triplicate determined of the samples as well as the automatically calculated both correlation coefficient and the relative standard deviation, which found to be 0.99998 and < 2 %, respectively. The measurement method (ICP-MS) was validated using working calibration solutions of the under-investigated metal ions (Pb, As, Se, Hg, Mn, Zn, Cd, Cr, and Ni). Standard solutions of all the investigated metals were prepared by stepwise dilutions of certified standard stock solutions. These solutions for calibration of all the metals investigated were prepared from ultra-grade a 1000 mg/L ICP multielement solution (Merck, Milan, Italy), using stepwise dilutions of certified standard stock solutions. The procedure validation included linearity, accuracy, and precision.

Sampling

In the present study, the samples were collected from Hail city, the northern province of Saudi Arabia using stainless steel scissors rinsed in ethanol. A total of 57 freshly cut collected samples (head hair) of volunteers across several occupational distributions at the age of 18 or above were divided according to the kind of smoking tobacco, stored at about 5°C until digestion and analysis processes. On the other hand, hairs samples were collected from volunteers divided into four groups: cigarette smokers, tobacco pipe (hookah) smokers, cigarette and hookah smokers, and non-smokers (control). The study was carried out in accordance with the Declaration of Helsinki and the protocol of research was approved by the Scientific Research Ethics Committee of the University of Hail, Saudi Arabia. Samples were collected from volunteers who were nonsmokers (control) for purpose of comparison. To obtained accurate data, we collected undyed hair samples for the present study. The collected samples were kept to seal tightly coded polythene bags in our laboratory for pre-treatment. Before sample collection, questionnaire surveys were performed for all participants containing highlights of information such as age, gender, educational level, water source, total monthly income, lifestyle, chronic diseases and/or drugs treatment, smoking habits, type of smokes, smoking period and number per day.

Results and Discussion

This study explains the risks of toxic and heavy metals coming from smoking of volunteers as well as the effect of second or third-hand smoke due to their families' smokers. In this context, the use of hair analysis for toxic and heavy metals has been recommended by the World Health Organization (WHO), due to the dependence of toxic and heavy metals levels in the hair on environmental contamination particularly smoking (Liang *et al*., 2017; Pozebon *et al*., 2017). Tobacco absorbs heavy metals mainly from soil or from the environment. So, smoking is one of the major sources of intake of toxic and heavy metals. In this work, we determine the concentration of toxic and heavy metals (Pb, As, Se, Hg, Mn, Zn, Cd, Cr, and Ni) in scalp hair samples of cigarette, hookah, and nonsmokers using the ICP-MS technique to estimate any relation between toxic and heavy elements concentration and smoking habitats. The ICP-MS measurement was validated by analysis of various standard samples and spiking known concentrations of toxic and heavy metals, were showed good accuracy, linearity, precision, and lower limit of detection. All measurements were carried out in triplicate and the mean values were automatically calculated. The metals investigated in this study were selected according to their toxicity and as well as their presence in smoked tobacco and therefore in the smoker's human body. Many researchers reported that there is a correlation between the high levels of toxic and heavy elements in hair and many dangerous human diseases such as cancer, mutagenicity, neurobehavioral decrements, cardiovascular diseases, gastrointestinal irritation, and immune dysfunction which maybe lead to deaths (Cabar *et al*., 2020).

Hair Analysis Results

In the current work, bio-monitoring of eight toxic and heavy metals was assessed using ICP-MS after microwave-assisted sample digestion with nitric acid in the whole hair samples (total n= 57) of some Saudi Arabia smokers and non-smokers. The volunteers were divided into two groups: non-smokers (n=18, ~32 %), and s smokers (n=39, ~ 68%). In this context, the smoker's participants were classified according to the kind of smokes into three sets, namely, cigarettes smokers (n= 27), tobacco pipe smokers (hookah), (n=7), and cigarettes + hookah smokers (n= 5). For comparison, the eight investigated metals were also determined under the same analysis conditions in whole hair samples of nonsmoker volunteers (n= 18).

Table 1 shows the values of investigated metals (Pb, As, Se, Hg, Mn, Cd, Cr, and Ni) obtained from the analysis of whole hair samples (n=18) of nonsmokers. The nonsmokers (control) volunteers were classified according to the ages as well as smoking state of the family as shown in **Table 1**. It was found that the concentration level ranges and averages of Pb, As, Se, Hg, Mn, Cd, Cr, and Ni were 125.7-2923.3 (683.9), 38.4- 144.8 (48.9), 95.9-298.0 (203.5), 32.8 - 626.8(189.0), 175.9 - 1788.5 (747.0), 4.3-68.9 (25.6), 139.7 - 2309.0 (943.5), and 178.4-2130.5(852.7), respectively. These obtained results are consistent with that reported for other parallel studies and are they are agreement the international tolerance levels (Alrobaian & Arida, 2019).

Table 1. Toxic and heavy metals levels in non-smokers (control) scalp hair (μg/kg)

No.	Age*, Year (y)	**Smoking state of the family		Hair dye	Pb	As	Se	Hg	Mn	Cd	Cr	Ni
		Yes	No									
1	a	Y	-	N	156.4	BDL	148.5	28.9	455.7	23.9	395.6	344.8
2	a	Y	-	N	988.7	38.8	221.6	48.9	722.0	13.8	577.6	188.5
3	a	N	-	N	1205.7	28.4	191.6	82.8	354.8	8.5	211.4	211.8
4	a	-	N	N	305.5	BDL	177.0	32.8	421.5	5.9	139.7	671.0
5	a	-	N	N	623.6	57.5	169.0	114.0	697.7	24.7	198.5	399.4
6	b	-	N	Y	125.7	89.9	274.8	189.8	1211.6	56.7	983.9	567.5
7	b	-	N	N	315.5	BDL	95.9	121.8	1521.0	32.1	732.6	432.8
8	b	Y		Y	699.8	76.0	254.7	231.5	1788.5	68.9	2012.4	1976.8
9	b	-	N	N	137.6	BDL	188.5	64.0	385.7	8.5	198.6	233.7
10	c	Y	-	N	378.0	53.7	231.8	187.8	544.7	9.7	2309.0	1708.0
11	c	Y	-	Y	722.0	144.8	208.9	626.8	898.6	32.7	1957.9	2130.5
12	c	Y	-	Y	2923.3	103.5	298.0	398.6	909.6	24.0	2131.5	898.6
13	c	Y	-	N	533.5	45.0	176.0	301.6	1232.8	15.0	465.8	1987.8

14	c	-	N	N	379.2	BDL	162.8	165.8	394.7	29.6	1211.3	975.0
15	e	-	N	N	396.7	55.7	231.4	57.0	175.9	44.7	1867.0	178.4
16	e	-	N	Y	411.6	80.9	196.5	98.6	655.7	53.0	757.8	1189.0
17	e	-	N	Y	2025.7	41.5	277.0	364.6	721.0	5.3	566.4	787.8
18	e	-	N	N	298.6	48.0	159.9	289.7	355.8	4.3	265.8	467.5

* Age: a = ≤ 21 y, b = 22-32 y, c = 33-43 y, e = ≥ 55 y.
** Yes = Y, No = N.
Hair dye: has dyed/bleached the hair during the last 3 years.

Within this context, the concentrations of toxic and heavy metals in biological samples like hair should be below and/or in good agreement with that of the international tolerance levels in so far as possible. Saudi men always wear a turban (shemagh) outside the house, which minimized exposure of their hair to outdoor contamination. This presented a good opportunity to evaluate the significance of the external pollutants. On the other hand, the relative fluctuation in the obtained values in each studied metal level reflects the variety in culture, age, environment, and habits. This is documented by the relative increase in the concentration of the studied metals in the hair samples of the smoking family compared with non-smoking families, as shown in **Table 1**.

Therefore, negative smoking has come also to play an important function in the exposure of nonsmokers to these toxic and heavy elements, that can be taken from the smoke through smoking. In other words, inhalation of tobacco smoke is a dominant source of heavy metals such as Cd, Ni, and Pb (Gönener *et al*., 2020). This is supported by comparing the toxic and heavy metals status of the nonsmokers according to their family smoking status, **Table 1**. Serdar et. al., (2012), reported that there is a positive relationship between the significant increase of toxic and heavy metals such as Cd, Pb, Cu, and Ni levels in hair and the frequency of smoking at the home of family members. Consequently, many factors contributed to introducing toxic and heavy metals into biological systems; this means the term of tolerant levels suitable for use instead of reference values.

Table 2 shows the concentration of toxic and heavy metal investigated in hair samples of 27 cigarettes smokers, Saudi participants. It was found that the concentration ranges and averages of Pb, As, Se, Hg, Mn, Cd, Cr, and Ni in the human hair of cigarettes smokers Saudi volunteers **(Table 2)** are 56.8-9899.8(2505.7), 43.8-370.3(176.9), 54.8-1311.6 (590.3), 37.8-341.7(196.9), 198.6- 1432.7 (731.6), 2.7-65.9(31.5), 138.9-2455.8(986.1), and 156.7-2219.8(916.6), respectively.

The data of nonsmokers **(Table 1)** and cigarettes smokers **(Table 2)** were compared. In general, it was noted that the concentrations of toxic and heavy metals were higher in cigarettes smokers than that in non-smokers, and this is in agreement with that reported by others for similar studies (Serdar et. al., 2012; Alrobaian & Arida, 2019). On the other hand, the concentrations of Pb, As and Se in nonsmokers were found to be significantly higher compared with them in cigarettes smokers, while the other element is nearly having the same levels in both cigarettes smokers and non-smokers. These results are consistent with other similar studies as well as below the international tolerance levels (Cabar *et al*., 2020). The concentrations of the investigated metals Pb, As, Se, Hg, Mn, Cd, Cr, and Ni) of 7 hookah smokers scalp hair volunteers were summarized in **Table 3**. Toxic and heavy elements values were compared according to the smoking kind consumption of the volunteers.

Table 2. Toxic and heavy metals levels in cigarettes smokers scalp hair (μg/kg)

No.	Age*, Year (y)	Smoking rate**	Pb	As	Se	Hg	Mn	Cd	Cr	Ni
1	a	2/2	445.7	52.8	69.6	42.8	311.5	3.7	205.9	832.7
2	a	1/2	56.8	43.8	57.5	45.7	198.6	2.7	138.9	321.8
3	a	3/4	977.0	169.6	121.0	39.8	544.8	32.8	299.6	466.8
4	a	2/5	59.8	280.6	539.7	176.9	463.8	22.8	988.8	744.0
5	a	4/5	1043.8	206.0	477.3	209.7	633.2	43.1	1109.6	688.7
6	a	3/3	598.7	311.6	422.4	88.5	1232.0	13.4	775.8	454.9
7	a	5/4	1121.5	280.6	396.7	68.9	833.7	35.9	1121.7	1434.8
8	b	11/4	4532.8	98.7	689.4	43.0	1012.8	45.8	886.8	1877.5
9	b	13/5	6879.8	314.8	832.5	146.8	699.2	21.8	322.0	2219.8
10	b	10/3	938.9	76.8	427.6	99.5	1232.8	2.7	422.9	266.8

11	b	9/6	1844.6	297.4	1077.6	235.8	1221.3	55.8	2213.0	743.8
12	b	8/3	875.8	142.0	279.5	158.9	336.8	42.7	644.9	322.4
13	b	10/6	9899.8	322.8	1232.7	69.8	1432.7	64.9	2321.7	1988.5
14	b	11/4	275.7	213.7	268.6	208.6	1331.3	58.6	1544.9	786.8
15	b	4/2	2192.7	73.7	89.9	37,8	656.7	4.9	188.7	199.5
16	b	7/4	1874.7	88.4	325.2	141.0	345.0	16.9	431.9	344.7
17	c	15/4	4532.7	203.0	432.6	286.8	877.6	33.2	745.9	2088.7
18	c	20/6	7432.7	166.5	1225.7	341.7	979.9	65.9	2310.7	2114.9
19	c	18/4	1243.0	134.0	985.9	308.3	544.7	36.8	1877.9	886.9
20	c	15/5	1321.7	91.8	974.7	266.0	798.6	29.9	661.9	233.7
21	c	8/2	479.8	69.7	54.8	86.3	765.8	46.8	233.9	156.7
22	e	22/4	1436.5	93,6	691.0	151.3	955.8	19.9	544.9	433.8
23	e	25/4	6743.6	262.4	873.4	271.2	779.5	38.9	779.9	342.8
24	e	27/5	8211.8	370.3	1137.8	279.9	887.9	43.9	998.5	1895.0
25	e	15/2	986.9	63.2	799.3	83.8	399.8	22.3	322.9	511.9
26	e	9/2	323.5	63.9	143.6	61.8	1311.7	7.3	189.7	833.9
27	e	35/5	1324.3	285.7	1311.6	247.6	799.7	16.9	2455.8	1556.9

* Age: a = ≤ 21 y, b = 22-32 y, c = 33-43 y, e = ≥ 55 y,

** Smoking rate = period of smoking (number of Years)/number of cigarettes per day).

Table 3. Toxic and heavy metals levels in hookah smokers scalp hair (μg/kg)

No.	Age*, Year (y)	Smoking rate**	Pb	As	Se	Hg	Mn	Cd	Cr	Ni
1	a	2/1	135.9	7.2	104.5	46.7	465.7	3.6	173.3	311.4
2	b	3/1	23.9	26.6	109.5	113.4	397.7	12.8	196.7	932.7
3	b	5/1	807.4	68.9	4768	446.3	2776.6	121.6	2698.4	887.9
4	c	4/2	1133.2	28.4	263.8	324.6	576.3	79.4	1987.9	1365.0
5	c	7/1	688.4	289.8	243.0	66.3	2865/9	236.5	6884.3	1964.6
6	c	5/1	969.8	112.4	345.3	321.5	1785.7	96.4	4537.2	1421.3
7	e	8/2	2198.5	267.4	734.6	521.3	3608.9	224.6	7988.5	2237.4

* Age: a = ≤ 21 y, b = 22-32 y, c = 33-43 y, e = ≥ 55 y,

** Smoking rate = period of smoking (number of Years)/number of hookah smoking per day).

It was found that a significant increase in the concentration values was obtained for all the eight investigated metals compared with that for nonsmokers **(Table 1)** or cigarettes smokers **(Table 2)** subjects. In this context, the average levels of Hg, Mn, Cd, Cr, and Ni were found to be slightly affected upon smoking cigarettes, while the averages of these metals levels were highly increased upon smoking hookah. In other words, the relatively significant fluctuation that occurred with data of all the investigated metals with the hookah smokers compared with cigarette smokers can be attributed to the completely different origin, manufacturing, and contents of cigarettes and hookah tobacco. The concentration ranges and averages of Pb, As, Se, Hg, Mn, Cd, Cr, and Ni in the hookah smokers scalp hair of Saudi volunteers **(Table 3)** are 23.9 - 2198.5(851.1), 7.2-289.8(114.4), 109.5-734.6(326.6), 46.7-521.3(262.9), 397.7-3608.9(1782.4), 3.6-236.5(110.7), 196.7-7988.5(3495.2), and 311.4-2237.4(1302.9), respectively.

To gain insight into the effect of both cigarettes and hookah smoking together on the concentration levels of the toxic and heavy metals in hair. The concentrations data of the investigated metals (Pb, As, Se, Hg, Mn, Cd, Cr, and Ni) of 5 cigarettes and hookah smokers scalp hair volunteers are summarized in **Table 4**.

The concentration ranges and averages of Pb, As, Se, Hg, Mn, Cd, Cr, and Ni in the cigarettes and hookah smokers scalp hair of Saudi volunteers **(Table 4)** are 148.7-12324.6 (5793.4), 10.3-480.3 (261.5), 523.4-1765.8 (1174.9), 68.9-524.2(296.5), 767.5-6100.4

(3711.8), 37.3-380.6(217.1), 406.4-10988.4 (4257.6), and 782.6-2138.4 (1852.3), respectively.

Table 4. Toxic and heavy metals levels in smokers (cigarettes + hookah†) scalp hair (μg/kg)

No.	Age*, Year (y)	cigarettes smoking rate**	Pb	As	Se	Hg	Mn	Cd	Cr	Ni
1	b	10/ 4	3509.3	78.4	523.4	113.6	767.5	45.8	406.4	1231.0
2	b	12/2	148.7	10.3	711.7	68.9	1469.3	37.3	983.6	782.6
3	c	15/4	9207.8	480.3	1332.0	321.5	4332.6	332.5	1055.0	2007.0
4	c	15/3	8776.7	275.0	1542.0	454.6	6100.4	288.9	7854.7	2138.4
5	e	23/2	12324.6	463.7	1765.8	524.2	5889.5	380.6	10988.4	3102.7

* Age: a = ≤ 21 y, b = 22-32 y, c = 33-43 y, e = ≥ 55 y,

** Smoking rate = period of smoking (number of Years)/number of smoking (cigarettes + hookah) per day).

† hookah = smoking hookah one time per day.

The impact of age and smoking period on element concentrations was investigated. We did not find any clear relation between the concentrations of heavy metals that influence age and smoking periods. However, further work is required in this area to clarify previous findings. Comparison for the average of each element in the four groups (non-smokers, cigarettes smokers, hookah smokers, and cigarettes+ hookah smokers) was performed as shown in **Figure 1**.

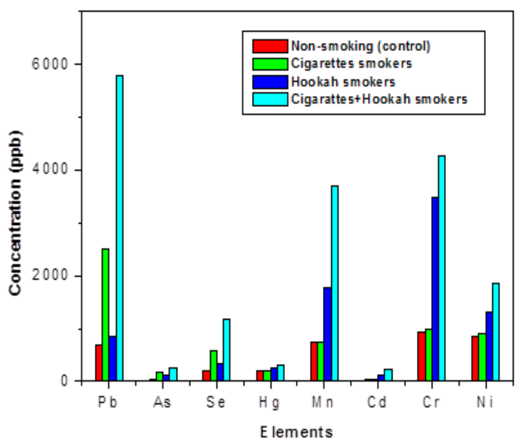

Figure 1. The average concentration of toxic and heavy metals in hair samples of 57 subjects of non-smoking and smoking (cigarettes and/or hookah) of Saudi volunteers.

In general, the sequences for the concentration of investigated metals in all groups (non-smoking, cigarettes smokers, hookah smokers and cigarettes+ hookah smokers) are the following: Cr> Ni > Mn > Pb >Se >Hg> As > Cd (non-smokers group); Pb > Cr >Ni> Mn > Se > Hg> As >Cd (cigarettes smokers group); Cr > Mn >Ni > Pb > Se> Hg > As ≥ Cd (hookah smokers); and Pb >Cr > Mn >Ni> Se> Hg> As> Cd (cigarettes+ hookah smokers), as shown in **Figure 1**. A comparison of the toxic and heavy metals concentrations in scalp hair of smokers and nonsmokers (control) volunteers was carried out by using Microsoft Excel of Office 365 to calculate the T-test. It was determined that didn't normal distribution, a value $p < 0.05$ was considered to be significant. As a result of this study, a statistically significant relationship has been found between smoking and the toxic and heavy metal levels in the hair of volunteers. Therefore, staying far from unhealthy behaviors like smoking can lead to a positive future life for smokers and their families.

In other words, these validated obtained results can support the efforts of the antismoking authorities.

Conclusion

The concentrations of the number of toxic and heavy metals (Pb, As, Se, Hg, Mn, Cd, Cr, and Ni) were determined by using ICP-MS in hair samples of 57 subjects of nonsmoking (control) and smoking (cigarettes and/or hookah) of Saudi volunteers. Passive smoking plays an important role in the increase of the investigated metals in the hair samples of nonsmokers and smokers. Overall, the levels of the studied metals in the hair of smokers were significantly high compared with those in non-smokers (control), and this was statistically significant, with regard to the smoking habit, for most the toxic and heavy metals. For the smoking period, the levels of Pb, As and Hg were significantly different but the levels of Mn, Cr, and Ni were not. This research shows hair as one of the most important marker valuable tissues for study to monitor the chemical toxicity introduced to the human body upon smoking. The obtained results revealed a correlation between the high levels of toxic and heavy elements coming from smoking, which supports the efforts of the antismoking authorities with validated data.

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