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Comparative Studies of Ni, Cd, Mn, Co, Pb, Cr and Zn in Hair, Nail and Plasma of Smokers and Non-smokers Subjects of Sargodha Zone

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Abstract

The present research work was focused to quantify and compare the level of most commonly exposed heavy metals in smokers and non-smokers subjects of different zones of Sargodha region. Heavy metals are generally considered and have been reported, play a very crucial role in destabilizing the immune system and producing carcinogenity. In this study we assessed and compared the level of Ni, Cd, Mn, Co, Pb, Cr and Zn in hair, nail and plasma samples of similar age group smokers and non-smokers. About 42 smoker and 42 non-smoker subjects were selected form Sargodha region, from which 21 were male and 21 female. Levels of the heavy metals were compared on different parameters including sex and age. Analysis was performed using Atomic Absorption Spectrophotometer (AAS). Total mean value of Zn was lower in smokers than non-smokers, whereas elevation of Cd, Mn, Co, Pb, Mn and Ni was observed in smokers as compared to non-smokers. Male smokers showed significantly ($p < 0.05$) higher values of metals than female. While the Smokers (male and female) whose age was less than 45 have lowers values of metals against the non-smokers of age > 45 years. However, lower concentration of Zn and Co was observed smokers of age > 45 years. The result showed significant impact of heavy metal accumulation via smoking on initiation of different fatal diseases in human beings. In conclusion such fatal diseases coulod be overcome by reducing exposure to heavy metals.

Key words: Heavy Metals, Hair, Nail, Plasma, Smokers, Non-smokers

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1. Introduction

Many adults and teenagers think that there are no effects of smoking on their bodies until they reach middle age [1]. 70% of adolescent smokers have already tried but failed to quit smoking and 90% of youths that smoke regularly suffers seriously strong cravings [2]. Cigarette is made up of tobacco, paper and additives. Usually in cigarette manufacture 600-1400 additives are used [3]. Cigarette smoking is responsible for more than 85% of lung cancers and also cause the mouth, pharynx, larynx, esophagus, stomach, pancreas, kidney, uterine, ureter, bladder and colon cancer. It has also been linked to Leukemia. Apart from the carcinogenic aspects of cigarette smoking, it is also linked to increased risks of cardiovascular diseases, cardiac arrest, peripheral vascular disease; sudden death and aortic aneurysm have also been established. Many components of cigarettes smoke irritate the lining of respiratory system characterized as Ciliotoxic materials. They cause increased bronchial mucus secretion and chronic decreases in mucociliary and pulmonary function [4]. The atomic absorption spectrophotometer is good technique used to analyze the presence of heavy metals [5-6]. Pakistan is an agricultural country, but only 0.27% of the total irrigated land is used for cultivation of tobacco in Pakistan. The annual production of tobacco is 70–75 million kg, whereas the domestic requirement is 40–50 million kg; the rest 30–35 million kg is exported. In most of the countries of the world the comsumption of cigarettes is falling while in Pakistan, the production and consumption of this nonessential poisonous item is increasing at an alarming rate. It is surprising that the world is fighting against smoking, an increasing number of Pakistani peoples setting new records by manufacturing an additional five billion cigarettes each year. The smoking rate among males is more than 50%, whereas it is 10% in females in Pakistan [7]. Heavy metals are in the water we drink, the air we breathe, the foods we eat, our daily household cleaners, our cookware and our other daily tools. Heavy metal poisoning

has become a major health problem, especially since the industrial revolution[8].

Material and Methods

The present study is analysis of heavy metals in the blood, hair and nails of smokers and then compares them with non-smokers.

2.1. Selection of subjects

Blood samples were taken from smoker and non-smoker subjects of different areas of Sargodha city (Fig. 1) and villages of Sargodha district (Fig. 2). There were 42 smokers and 42 non-smokers including both gender with similar age group. Among smokers 24 were males and 18 were females and likewise for control subjects age matched in 20-80 years age range. A structured questionnaire was used to obtain necessary data on occupational, physical activity, life style pattern as smoking or non-smoking habit. From each patient and control subjects three types of biological samples were collected namely fresh blood, scalp hair and nails (foot and hands).

2.2. Blood samples collection

Blood samples (5 ml) were collected from subjects via vein-puncture with sterilized syringe for determination of heavy metals. Na_2EDTA powder in small amount was sprinkled inside the walls of labeled glass vials and blood was stored in it. All blood vials were kept in refrigerator at $10^{\circ}C$ until for further analysis.

2.3. Hair samples collection

Hairs (less than 0.5 g) of smokers and non-smokers were cut at the root of occipital area with stainless steel scissors and stored in labeled polyethylene bags. Finally all of these samples were kept in plastic baskets and brought to laboratory for analysis.

2.4. Nails samples collection

Nails (1 g) of smokers and non-smokers were cut with stainless steel nail cutter. Nails were stored in polyethylene bags having lock at its mouth. Each bag was labeled to avoid any misperception. All bags were kept in plastic basket and brought to laboratory for further analysis.

2.5. Pretreatment of samples

2.5.1. Washing of samples

In order to remove dust, grease, gel, and oil from hairs and dust or polish from nails, samples were washed thrice with acetone followed by water and again with acetone as recommended by IAEA Advisory Group, followed by drying in electric oven at 60^0C. [9].

2.5.2. Blood samples preparation for analysis

Plasma from blood was isolated by centrifugation at 1000 rpm. From each sample measured plasma was isolated by micro pipette and volume of each plasma sample was recorded. This plasma was transferred to digestion flask. In each flask, 5 mL of 60% HNO_3 was added and kept overnight. 2 mL of H_2O_2 was added into each and kept on hot plate at $180^{\circ}C$ for 2-3 minutes. When white fumes started evolving, it was completion of digestion process. Volumes of all samples were made up to 15 ml with doubly distilled de-ionized water in sample vials [9].

2.6. Hair samples preparation for analysis

One gram of hair taken from each individual was cut into small pieces. Conventional wet acid digestion method was applied in which each sample was soaked in ultrapure nitric acid (5 ml, 60%) overnight followed by addition of H_2O_2 (1 ml) and digested on hot plate at $180^{\circ}C$ until white fumes started evolving, which showed completion of digestion process [10]. Volumes of all samples were made up to 20 ml by doubly distilled de-ionized water and stored in sample vials for further analysis.

2.7. Nails samples preparation for analysis

One gram of each nails sample we exactly weighed with electrical analytical balance and cut into small pieces and then put into labeled digestion flask (100ml). And each was soaked in ultrapure nitric acid (5 ml, 60%) overnight. Then one ml H_2O_2 was added and kept on hot plate at $180^{\circ}C$ for 2-3 minutes until white fumes started evolving. It was indication of completion of digestion. Volumes of all samples were made up to 20ml by addition of doubly distilled de-ionized water and samples were stored in clean labeled samples vials for further analysis.

2.8. Preparation of standard solution

Standard solutions were prepared by using doubly distilled deionized water. Amount of salt required for 1000 ppm solution. From this 1000 ppm solutions further aliquots (100ppm, 10ppm, 3ppm, 2.5ppm, 2.0 ppm, 1.5 ppm, 1.0 ppm, 0.5 ppm, 0.1 ppm, 0.05 ppm, 0.01 ppm, 0.001 ppm) were prepared.

2.9. Analysis of Samples

For analysis of all samples Flame Atomic Absorption Spectrophotometer (FAAS) was employed. It is considered as one of the most effective, modern and completely economical instrument for routine work analysis [11].Different specification used in instrument during analysis of Ni, Cr, Mn, Zn, Co, Cu and Cd are shown in Table 1.

3. Results and Discussion

Table 2 shows results for smokers and non-smokers used in the study. In the above distribution 50% were male while 50% were female. Male patients were selected according to different age groups such as 23.8% male were at the age of 20-40 and 47.62% were at the age of 42-60 and 28.57% were at the age of 60-80 years old. While 71.42% female patients were at the age of 25-40 and 28.57% were 40-60 years old. Whereas non-smokers subjects of males and females have the same ratio. In present work analysis of some heavy metals like Cd, Cr, Co, Pb, Mn, Ni and Zn have been carried out in hair, nail and plasma of the smokers against the control subjects. The values listed in Table 3 are the mean values ± standard deviation of the smokers Vs non-smokers.

Table 1: Parameters used for measurement on Flame Atomic Absorption Spectrophotometer (Schimadzu Japan AA-6300)

Metals	Wave length	Slit width	Lamp mode	Lamp current low	Lamp current high	Burner height	Burner angle	Flame type	Fuel gas	Support gas
Cd	228.8nm	0.7nm	BDC-D2	8 mA	0 mA	7 mm	0°C	Air+C_2H_2	1.8 L/min	15 L/min
Co	240.7nm	0.2nm	BDC-D2	12 mA	0 mA	7 mm	0°C	Air+ C_2H_2	1.6 L/min	15 L/min
Fe	248.3nm	0.2nm	BDC-D2	12 mA	0 mA	9 mm	0°C	Air+ C_2H_2	2.2 L/min	15 L/min
Cu	324.8nm	0.7nm	BDC-D2	6 mA	0 mA	7 mm	0°C	Air+ C_2H_2	1.8 L/min	15 L/min
Mn	279.5nm	0.2nm	BDC-D2	10 mA	0 mA	7 mm	0°C	Air+ C_2H_2	2 L/min	15 L/min
Ni	232.0nm	0.2nm	BDC-D2	12 mA	0 mA	7 mm	0°C	Air+ C_2H_2	1.6 L/min	15 L/min
Zn	213.9nm	0.7nm	BDC-D2	8 mA	0 mA	7 mm	0°C	Air+ C_2H_2	2 L/min	15 L/min
Pb	283.3nm	0.7nm	BDC-D2	10 mA	0 mA	7 mm	0°C	Air+ C_2H_2	2 L/min	15 L/min
Mg	285.2nm	0.7nm	BDC-D2	8 mA	0 mA	7 mm	0°C	Air+ C_2H_2	1.8 L/min	15 L/min
Ca	422.7nm	0.7nm	BDC-D2	10 mA	0 mA	7 mm	0°C	Air+ C_2H_2	2 L/min	15 L/min

Table 2: Descriptive data for smokers and non-smokers.

Gender and Age	Smokers (%)	Non Smokers (%)
Male	21/42 (50%)	21/42 (50%)
Female	21/42 (50%)	21/42 (50%)
Male		
20-40	5/21 (23.8 %)	5/21 (23.8 %)
42-60	10/21 (47.62%)	10/21 (47.62%)
60-80	6/21 (28.57%)	6/21 (28.57%)
	Female	
30-44	15/21 (71.42%)	15/21 (71.42%)
45-60	6/21 (28.57%)	6/21 (28.57%)

Table 3: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Smokers and Non smokers

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Smokers (N=42)	Plasma	7.08±0.60	32.28±0.26	9.06±0.62	10.86±0.24	4.32±0.48	3.97±0.45	95.79±1.4
	Hair	17.04±0.6	2.58±0.33	18.45±0.69	21.04±0.66	4.19±0.38	1.79±0.38	92.08±1.8
	Nail	17.65±0.47	2.45±0.32	19.77±0.89	23.56±0.31	2.78±0.54	3.36±0.63	88.45±1.6
Non Smokers (N=42)	Plasma	6.34±0.36	2.01±0.37	11.52±0.11	5.94±0.96	2.43±0.43	2.06±0.46	112.8±1.5
	Hair	8.95±0.45	1.89±0.21	5.85±0.45	19.74±0.18	1.82±0.28	1.07±0.58	98.21±1.7
	Nail	2.75±0.08	1.81±0.09	15.03±0.02	9.52±0.44	1.37±0.05	1.56±0.41	101.01±1.8
p_{Value}	Plasma	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05
	Hair	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05
	Nail	p<0.05	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 4: Concentration (µg/g for Hairs and Nails, µg/mL for Plasma Samples) of Smokers (Men Vs Women)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Smoker men	Plasma	7.45±0.46	2.47±0.14	12.18±0.85	10.32±1.48	4.74±0.67	4.05±0.36	88.51±2.34
	Hair	18.94±1.46	1.57±0.23	22.69±7.03	13.72±5.01	1.98±0.83	2.06±0.46	98.18±2.43
	Nail	19.24±1.16	4.46±1.47	23.07±2.48	7.38±1.24	2.39±0.64	3.73±0.43	93.52±3.31
Smoker Women	Plasma	6.61±0.33	2.07±0.21	10.89±0.95	9.46±0.55	2.87±2.01	3.57±0.31	100.5±22
	Hair	14.64±3.89	0.59±0.43	4.49±1.38	8.48±2.56	1.66±0.34	1.55±0.17	95.36±5.09
	Nail	16.08±2.17	0.43±0.29	16.23±6.59	5.26±1.33	1.87±0.08	2.78±0.55	89.92±12.9
p_{Value}	Plasma	p<0.05	p>0.05	p<0.05	p<0.05	P<0.05	p<0.05	p<0.05
	Hair	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05	p<0.05
	Nail	p<0.05	p<0.05	p<0.05	p<0.05	P<0.05	p<0.05	p<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 5: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Non Smokers (Men Vs Women)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Non-smoker Women	Plasma	5.55±1.07	1.79±0.31	7.84±3.44	5.69±1.09	1.21±0.35	1.42±0.41	101.01±19
	Hair	8.95±8.45	0.38±0.24	14.49±2.96	9.23±6.32	0.96±0.66	0.46±0.35	98.23±5.62
	Nail	2.77±0.06	1.17±0.07	9.18±5.76	2.56±0.53	1.01±0.75	1.04±0.12	93.74±12.7
Non-smoker Men	Plasma	7.24±0.65	1.79±0.49	10.16±1.49	6.17±0.82	1.63±0.25	1.88±0.42	104±22.52
	Hair	15.6±4.42	0.31±0.26	7.88±6.56	11.43±5.43	1±0.69	1.23±0.32	97.53±7.11
	Nail	1.08±0.14	1.41±0.48	12.08±7.28	3.18±0.98	1.11±0.14	1.59±0.200	92.99±8.82
p_{Value}	Plasma	p<0.05	p>0.05	p<0.05	p>0.05	p>0.05	p>0.05	p<0.05
	Hair	p<0.05	p>0.05	p<0.05	p<0.05	p>0.05	p<0.05	p<0.05
	Nail	p<0.05	p>0.05	p<0.05	p<0.05	p>0.05	p>0.05	p<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 6: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Smokers (men) Vs Non Smokers (men)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Smoker Men	Plasma	7.45±0.46	2.47±0.14	12.18±0.85	10.32±1.48	4.74±0.67	4.05±0.36	88.51±2.34
	Hair	18.94±1.46	1.57±0.23	22.69±7.03	13.72±5.01	1.98±0.83	2.06±0.46	98.18±2.43
	Nail	19.24±1.16	4.46±1.47	23.07±2.48	7.38±1.24	2.39±0.64	3.73±0.43	93.52±3.31
Non-smoker Men	Plasma	7.24±0.65	1.79±0.49	10.16±1.49	6.17±0.82	1.63±0.25	1.88±0.42	104±22.52
	Hair	15.6±4.42	0.31±0.26	7.88±6.56	11.43±5.43	1±0.69	1.23±0.32	97.53±7.11
	Nail	1.08±0.14	1.41±0.48	12.08±7.28	3.18±0.98	1.11±0.14	1.59±0.200	92.99±8.82
p_{Value}	Plasma	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
	Hair	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05	p>0.05	p>0.05
	Nail	P<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 7: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Smokers (Women) Vs Non Smokers (Women)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Smoker Women	Plasma	6.61±0.33	2.07±0.21	10.89±0.95	9.46±0.55	2.87±2.01	3.57±0.31	100.5±22
	Hair	14.64±3.89	0.59±0.43	4.49±1.38	8.48±2.56	1.66±0.34	1.55±0.17	95.36±5.09
	Nail	16.08±2.17	0.43±0.29	16.23±6.59	5.26±1.33	1.87±0.08	2.78±0.55	89.92±12.9
Non-smoker Women	Plasma	5.55±1.07	1.79±0.31	7.84±3.44	5.69±1.09	1.21±0.35	1.42±0.41	101.01±19
	Hair	8.95±8.45	0.38±0.24	14.49±2.96	9.23±6.32	0.96±0.66	0.46±0.35	98.23±5.62
	Nail	2.77±0.06	1.17±0.07	9.18±5.76	2.56±0.53	1.01±0.75	1.04±0.12	93.74±12.7
P_{Value}	Plasma	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05
	Hair	P<0.05	P>0.05	P<0.05	P>0.05	P>0.05	P<0.05	P<0.05
	Nail	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 8: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Smokers (Women< 45) Vs(Women > 45)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Smoker Women<45	Plasma	4.73±0.45	1.65±0.71	7.62±1.94	8.54±1.27	1.59±0.12	1.57±0.38	99.01±11.4
	Hair	11.46±2.98	0.43±0.12	3.94±1.21	8.38±1.29	1.29±0.71	1.38±0.08	95.38±7.01
	Nail	12.05±3.12	0.34±0.08	12.46±1.33	4.98±0.33	1.78±0.11	2.59±0.55	89.07±13.5
Smoker Women>45	Plasma	6.21±0.52	2.04±0.31	9.54±0.55	6.46±0.55	2.87±2.01	2.95±0.31	89.5±22
	Hair	13.89±3.64	0.59±0.21	4.31±1.21	7.48±2.56	1.66±0.34	2.75±0.17	93.36±5.09
	Nail	15.17±1.37	0.45±0.28	14.56±4.51	2.96±1.33	1.87±0.08	2.78±0.55	91.92±12.9
P_{Value}	Plasma	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05
	Hair	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05	P<0.05
	Nail	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05	P>0.05	P<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 9: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Smoker (Men <45) Vs Smoker (Men>45)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Smoker Men<45	Plasma	5.73±0.45	1.65±0.71	7.62±1.94	9.54±1.27	1.59±0.12	2.01±0.05	100.91±11.4
	Hair	13.46±2.98	1.13±0.12	13.94±1.21	12.38±1.29	1.29±0.71	1.32±0.38	95.38±7.01
	Nail	15.05±5.12	2.36±0.91	17.46±1.33	7.98±0.33	1.78±0.11	2.59±0.55	91.07±13.5
Smoker Men>45	Plasma	7.21±0.52	2.04±0.31	11.54±2.55	6.46±1.55	4.27±2.01	3.27±0131	99.5±22
	Hair	17.89±3.64	1.59±0.21	21.31±1.21	7.48±2.56	1.66±0.34	2.05±0.17	98.36±5.09
	Nail	18.17±1.37	3.82±1.28	22.56±3.51	4.06±1.33	2.07±0.28	3.08±0.55	94.92±12.9
P_{Value}	Plasma	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
	Hair	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05
	Nail	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 10. Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Non Smoker (Women<45) Vs Non-Smoker (Women>45)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Non Smoker Women<45	Plasma	3.55±1.07	1.09±0.31	5.84±3.44	5.99±1.09	1.21±0.35	1.35±0.41	101.01±13.1
	Hair	6.95±2.45	0.38±0.24	11.49±2.96	9.83±6.32	0.89±0.66	0.46±0.35	98.23±7.62
	Nail	1.77±0.06	1.17±0.07	7.18±5.76	2.01±0.53	1.01±0.75	1.04±0.12	93.74±12.7
Non Smoker Women>45	Plasma	5.55±1.07	1.79±0.31	7.84±3.44	3.69±1.09	1.21±0.35	1.42±0.41	99.01±19
	Hair	8.95±8.45	0.38±0.24	14.49±2.96	7.23±6.32	0.96±0.66	0.46±0.35	98.23±5.62
	Nail	2.77±0.06	1.17±0.07	9.18±5.76	2.56±0.53	1.01±0.75	1.04±0.12	96.74±12.7
P_{Value}	Plasma	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05
	Hair	P<0.05	P>0.05	P<0.05	P<0.05	P>0.05	P<0.05	P>0.05
	Nail	P<0.05	P>0.05	P<0.05	P>0.05	P>0.05	P<0.05	P<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

Table 11: Concentration (µg/g for Hairs & Nails, µg/mL for Plasma Samples) of Non Smoker (Men < 45) Vs (Men > 45)

	Samples	Ni	Cd	Mn	Co	Pb	Cr	Zn
Non-smoker Men<45	Plasma	6.24±0.65	1.39±0.49	8.16±1.49	6.17±0.82	1.13±0.25	1.08±0.42	99±17.52
	Hair	12.6±4.42	0.71±0.26	6.88±4.56	11.43±5.43	0.44±0.69	0.64±0.32	93.53±7.11
	Nail	1.98±0.14	1.25±0.48	9.08±6.28	3.18±0.18	0.57±0.14	1.17±0.20	97.99±8.82
Non-smoker Men>45	Plasma	7.04±0.65	1.79±0.49	10.16±1.49	5.17±0.82	1.63±0.25	1.98±0.42	94±13.52
	Hair	14.6±4.42	0.98±0.26	7.88±6.56	8.43±5.43	1.21±0.69	1.53±0.32	89.53±7.11
	Nail	2.08±0.14	1.41±0.48	12.08±7.28	1.18±0.98	1.41±0.14	2.30±0.200	92.05±8.82
P_{Value}	Plasma	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
	Hair	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
	Nail	P>0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

t-Test Analysis (p<0.05=Significant Difference, p>0.05=Non-Significant Difference)

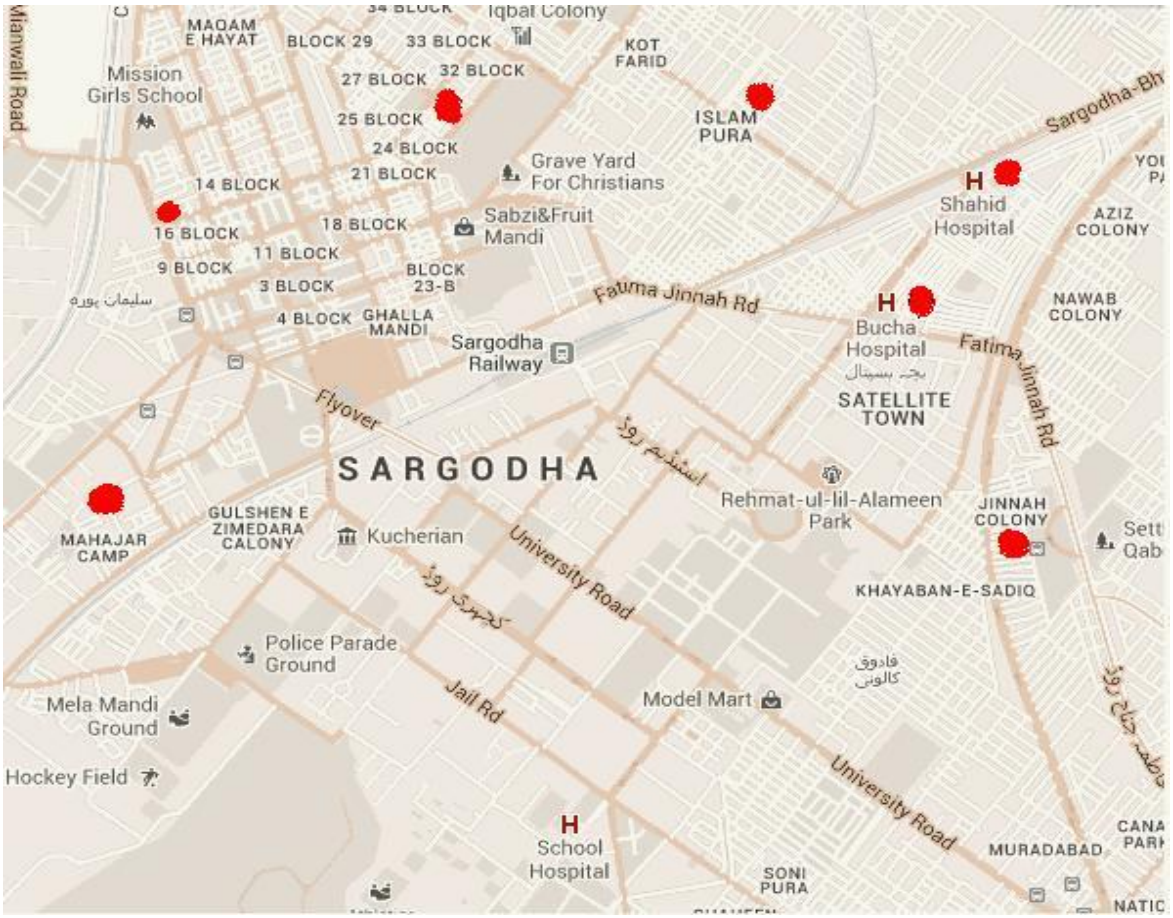

Fig. 1. The Sargodha city areas selected for collection of samples

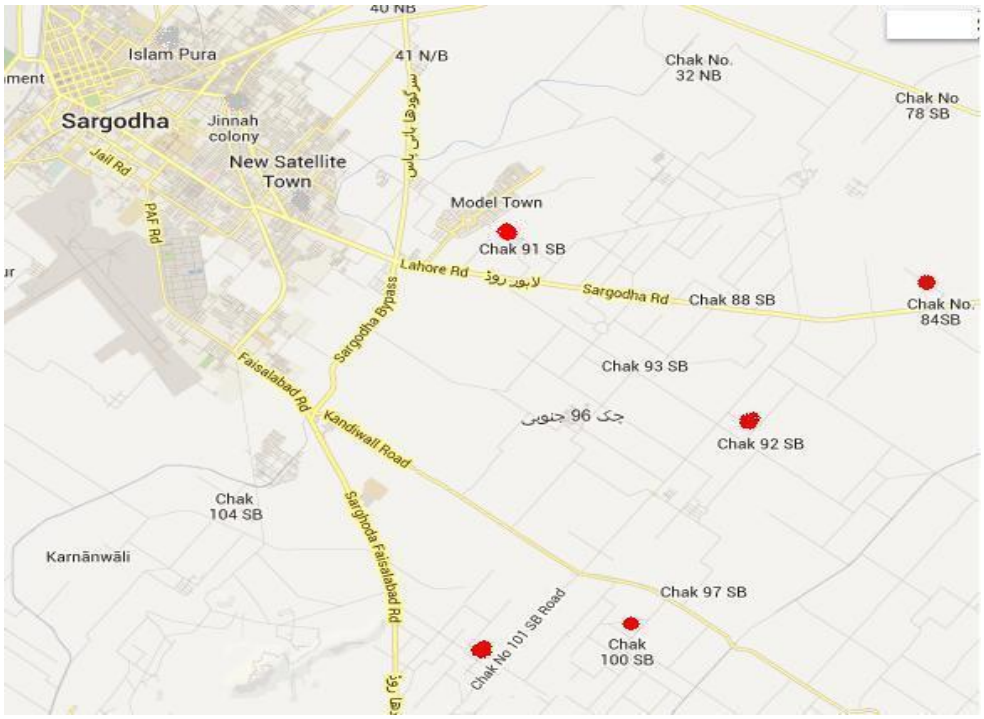

Fig. 2. The Sargodha region areas selected for collection of sa

As data acquisition for Ni, Co, Mn, Pb and Zn in smokers and non-smokers shows the significant difference, but some non-significant difference is found in case of Cd hair and nail samples and also for the plasma and hair samples of Cr. In Table 4 values of metals are compared between smoker men and women. It is found that in case of Co, Ni, Mn, Cr and Zn significant difference is found in all types of samples, but in case of Cd plasma samples shows non-significant difference and in case of Pb hair samples shows non-significant difference. Although smoker men have relatively high concentration of all metals as compared to smoker women but in some cases difference is significant while in others it is not. By comparing metal concentration in Non-Smoker men and women, Ni, Mn, Zn shows significant difference in all biological samples, while Cd and Pb shows non-significant difference in all biological samples. In case of Co there is non-significant difference in plasma samples and in Cr hair samples shows the significant difference. Same results were obtained for smoker Men Vs Non-Smokers Men as found in the preceding Table 5. While their statistical values in the table 5 shows significant and non-significant data. Table 6 shows that Mn and Co shows significant difference in all biological samples, while plasma samples of Ni and Cd shows non-significant difference, and hair samples of Pb, Cr and Zn also shows non-significant difference. By comparing the levels of metals in Smoker Women and Non-Smokers Women, Ni, Mn, and Cr shows significant difference in all biological samples, while Cd shows non-significant difference in all biological samples. In case of Co there is non-significant difference in hair samples. In Zn hair and nail samples shows the significant difference, while in Pb hair and nail samples shows the non-significant difference. The results showed in the above table 7 show that concentration of heavy metals is higher in Smoker Women as compared to the Non-Smoker Women, which have low concentration of heavy metals. While concentration of Mn in hair samples of Non-Smoker Women is high as compared to the Smoker Women. Age wise comparison of the metals in the Smoker Women, significance difference (p <0.05) is observed in case of nickel and zinc, while non-significant difference (p> 0.05) is observed in case of cadmium and lead. In case of manganese and Co there is no significant difference in hair samples, while in case of Cr nail samples have no significant difference. This difference is easily observed Table 8. The results showed that concentration of heavy metals is higher in Smoker Women>45 years as compared to the Smoker Women<45 years, which have low concentration of heavy metals. Zn and Co have low concentration in women>45 (Table 8). Age wise comparison of the metals in Smoker Men, significance difference (p <0.05) is observed in case of Ni, Mn, Co, Cr and Zn, while non-significant difference (p> 0.05) is observed in case of Pb and Cd hair sample (Table 9). The results when compared the graphs shows that concentration of heavy metals is higher in Smoker Men>45 years as compared to the Smoker Men < 45years, which have low concentration of heavy metals. While concentration of Co and Zn is higher in Smoker Men < 45 (Table 9). Non-Smoker Women< 45 years Vs Non-Smoker Women>45 years when compared than it is concluded that almost all the metals have high levels in Non-Smoker Women>45 except Co and Zn which have low level. This difference can easily be observed by plotting their comparative in Table 10. From results, it is procured that high concentration of metals is observed in Women who are above 45 years. While in case of Zn and Co it is seen that concentration of heavy metals is lower in Non-Smoker Women who are greater than 45. Age wise comparison of the metals in the Non-Smoker Men, significance difference (p <0.05) is observed in case of Mn, Co, Pb, Cr and Zn, while non-significant difference (p > 0.05) is observed in case of Cd. In case of Ni there is no significant difference in nail samples (Table 11). When compared the concentration of heavy metals is higher in Non-Smoker Men > 45 years as compared to the Non-Smoker Men < 45 years, which have low concentration of heavy metals. While in case of Co and Zn the concentration of metals is lower in Men > 45 (Table 11).

The present work focuses the analysis of some of the heavy metals (Zn, Pb, Cr, Co, Mn, Ni and Cd) in 3 types of biological samples *i.e.* hairs, nails and plasma of the Smokers against the non-smoker persons (Tables 1-11). Emphasis to this work is only the estimation of mentioned heavy metals in the collected samples, while their mechanism of action is not of our interest, only their levels are sight out that how smoking effect the concentration of these heavy metals in human body. There are no safe levels of lead within the body and the threshold for Pb levels in the blood has been reviewed over the past several years. On the basis of different epidemiological studies, it has been recommended that levels of blood Pb should be kept below 10 µg/dL. However, it has been reported that blood Pb level of even 2 µg/dL are associated with high rates of cardiovascular disease (CVD) and Pb level of 3.6 µg/dL could be responsible for 89% increase in mortality from cardiac disease. The mechanism behind this deleterious effect of Pb is not clear; however blood Pb levels greater than 10 µg/dL have been considered to be responsible for increased rate of arteriosclerosis [12]. Our present study also shows that the smokers have high concentration of lead in plasma as compared to the non-smokers, it is confirmed by World Health Organization that smoking of 20 cigarettes per day has been estimated to result in the inhalation of 2–4 µg 1–5 µgPb, or even more [13]. Women have lower concentration of lead level in our data than that of men, same findings have been reported by [14]. Pb concentration in the blood of the cigarette smokers (52.12 µg/l) was higher by 29% than in the non-smokers (40.42 µg /l) [15], which is best agreement with our present study in which smokers have concentration of Pb (4.32 µg) while non-smokers (2.43 µg).

The present study shows that the smokers have low level of Zn as compared to non-smokers. Also the males (patients and normal) have high concentrations of zinc in all types of samples which are similar to the results mentioned above, but [16], has reported higher values of zinc in females than males which is contradictory to the present study. Zinc deficiency is frequent in the old age, which is in good agreement with our findings as in the Tables 8-11 which shows the metal values related to the ages of male and females (smokers & non-smokers). Same trend was found in the hair and nail samples of smokers and non-

smokers, while not enough literature is available to compare. The present study shows that smokers have high concentration of Mn as compared to the non-smokers. Our data also shows that men have high concentration of Mn than that of women, which is good agreement when it is compared [17], where women required 1.8 milligram, while men required 2.3 milligram.

The level of chromium in mainstream cigarette smoke ranges from 0.0002 – 0.5 mg per cigarette [18]. In above work it is found that concentration of Cr is higher in biological samples of smokers, while in non-smokers concentration is low in all biological samples. It was also seen that with the increase in age the concentration of metal also increased. Our this outcome supported [19], in which concentrations of about 4.3 mg/kg (dry weight) are found in smokers compared to 1.3 mg/kg in non-smokers, it increased with age and smoking time.

The values of nickel in observed serum samples are relatively higher in smokers than non-smokers, it is supported by [20], who analyze the concentration of nickel in blood of smokers and non-smokers. They found nickel content in the blood of smokers (0.01-0.42 µg/l) was higher than in the blood of non-smokers (0.01-0.26 µg/l). Higher values of nickel in hair and nails are obtained but no literature is available to compare our work in these parameters except serum levels. Smokers can have twice cadmium in their blood as compare to non-smokers. Studies showed that the amounts of cadmium present in tobacco smoke are capable of affecting our health. It can damage the kidneys and the linings of the arteries and is a known cause of different types of cancer [21]. Higher plasma cadmium values are obtained in smokers than non-smokers, also smoker women have higher values as compared to non-smoker women, it is also found that nail and hair samples also have high concentration of Cd, and our findings are very much comparable with [16], who also obtained same kind of results in the above mentioned parameters.

Cobalt has little direct activity in the body; it is a vital component of Vitamin B12. Deficiency of vitamin B_{12} can lead to a number of diseases, including heart disease, diarrhea, mental disturbances and various other nervous system dysfunctions [22]. In our findings relatively high values of cobalt are obtained in all types of biological samples and men (smokers and non-smokers) have relatively higher values of cobalt than women respectively, but when we compare them with age factor then it was seen that there is low concentration of cobalt in old age in all biological samples.

4. Conclusion

From this study the levels of heavy metals was assessed in different blood samples of males and females smokers and non-smokers in different zones of Sargodha region. A strong relationship was found between smokers and these metals, when samples of smokers and non-smokers were compared. Significant difference was found in case of each metal. Some of the metals showed deficiency in case of smoking, while levels of some trace elements were elevated. This deficiency/excess of certain metals under study may be due to a number of factors contributing to minimize or increase these metals in human body when they smoke, their diet, environmental exposure, way of living, professions, age and sex also. Our findings are in good agreement with the previous literature data, yet so much work is needed in order to avoid the prevalence of smoking in Pakistan. The correct mechanism of some of the metals, which effect human body by smoking, is not known yet. So, special attentions are required with the aid of different research organizations to highlight this area.

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