Environmental effect of pollution and smoking on protein, cholesterol, albumin and bilirubin

[a]Zafar Iqbal, [a]A.H. Bangash, [b]Ihsanullah, [c]H. Rasheed and [a]S. Zakir

[a]Department of Pharmacy, University of Peshawar, Pakistan
[b]Nuclear Institute of Food & Agriculture, Peshawar, Pakistan
[c]Department of Pharmacy, University of Peshawar, Pakistan

Abstract

In order to see the effect of pollution and smoking, blood samples of 153 healthy adult men volunteers were collected from polluted and non-polluted areas of Peshawar. The age, height, duration and stay in the specific area and smoking habits were recorded. Serum was analyzed for protein, cholesterol, albumin and bilirubin. The data revealed that pollution and smoking both have no significant effect on the level of cholesterol while smoking has prominent effect on the level of total protein. The results show that pollution has a prominent effect on serum albumin i.e. the level of serum albumin was higher in smoker living in non-polluted area compared to the polluted area. From the observations, it is clear that pollution has altered the level of bilirubin and significant difference was observed in smokers and non-smokers living in polluted and non-polluted area. The data also indicate that the level of bilirubin was higher in volunteers living in non-polluted area than polluted area.

1. Introduction

Environmental pollution is a current burning issue. Pollution has directly adverse effect on human beings. The magnitude of the problem of air pollution has increased alarmingly due to population explosion, industrialization, urbanization, automobiles and other human proclivities for greater comfort. It is important to say that many chemicals released into the air may react, producing additional secondary pollutants (1). In an epidemiological study conducted in Hong Kong, the relation between air pollution and health was reviewed (2). The results of another study suggest that occupational exposure to vehicle emissions may induce detectable adverse health effects (3). Coal fly ashes (CFA) contain toxic constituents like metals, polycyclic aromatic hydrocarbons and silica (4). Air pollution is a complex mixture of small and large particles of varying origin and chemical composition. Small particles, such as those from fossil fuel combustion, are more dangerous, because they can be inhaled deeply into the lungs. Similarly, environmental tobacco smoke (ETS) is a dynamic, complex mixture of more than 4,000 toxic or carcinogenic chemicals found in both vapor and particle phases and is a cause of lung, emphysema, chronic obstructive pulmonary diseases, cardiovascular and other diseases (5). In tobacco smoke products (TSP) induced pulmonary disease has been hypothesized. Carbon monoxide is considered as an asphyxiant. (6). Sources of biological air pollutants include outdoor air include viruses and bacteria, animal occupants (insects and other arthropods, mammals) that shed allergens, and indoor surfaces and water reservoirs where fungi and bacteria can grow, such as humidifiers. Biological agents in air are known to cause three types of human diseases such as infections, hypersensitivity and toxicosis (7). Health professionals are well aware of the threat from toxic lead, mercury

vapor and radon. In addition, volatile organic compounds (e.g., formaldehyde, benzene, perchloroethylene etc.) are emitted as gases from certain solids or liquids (8). Some pesticide having active ingredients and inert components are considered possible human carcinogens (9).

Peshawar is the capital of North West Frontier Province and is densely populated. The influx of Afghan refugees has further aggravated the problem in this city. Urban air in the Peshawar City is polluted mainly due to vehicular exhausts and emissions from the large number of small-scale brick kilns factories. The aim of present study was to evaluate effect of pollution and smoking on serum protein, cholesterol, albumin and bilirubin.

2. Materials and method

2.1 Instrumentation

a) Microlab 200: automatic spectrophotometer, (Merck; Germany).
b) Centrifuge Model Hermle Z-232, China (1000–5000 rpm).
c) Portable and battery operated gas detector.
d) Water bath Model is WF-200 Gallenkamp (England).

2.2 Method of sampling

a) Selection of volunteer: One hundred fifty three **a**dult, healthy adult volunteers from Ramdas Bazar, Khyber Bazar, New Bus Stand, Hayatabad, and Harichand, living for at least for 6 months in those particular areas were selected randomly. It was not possible to collect samples from female. To see whether smoking does have some effects on liver functions, the volunteers were further divided into two groups i.e. smokers and non-smokers. Here smokers were defined as that one who smokes regularly any brand of cigarette.
b) Blood sampling: Blood (5 ml) was obtained randomly from the cephalic vein of the volunteer by using a 5 cc disposable syringe. The blood was transferred to the glass test tube and kept in a dark for 30 minutes to clot. The samples were centrifuged at 3000 rpm for 5 minute and serum was collected. The age and duration of stay in the specific area were also recorded.

2.3 Method of analysis

The blood serums were analyzed for various tests using kit methods. Details of relevant information and reagents are given below:

	Protein	**Cholesterol**	**Albumin**	**Bilirubin**
Manufacturer	Clonital	Clonital	Clonital	Merck
Country	Italy	Italy	Italy	Germany
Normal values	6.6-8.3 g/dl	150-240 mg/dl	3.5-5.0 g/dl	Upto 1 mg/dl
Linearity of method	Upto 12 g/dl	Upto 700 mg/dl	Upto 6 g/dl	10.5 mg/dl
Reagents	**Biuret Reagent**: Sodium potassium tartrate	Phosphate buffer Cholesterol oxidase Cholesterol esterase	**BCG Reagent**: Citrate buffer Bromocresol	**R1**: Sulfanilic acid **R2**: Sodium nitrite **R3**: Accelerator

Cupric sulfate Sodium hydroxide Potassium iodide Protein standard	Hydroxybenzoic acid 4-aminophenazone Peroxidase Surface active agents Stabilizers Cholesterol standard	Triton X-100 Albumin standard	(caffeine, benzoate & sodium acetate) **R4**: Fehling solution II

Total Protein (Biuret Method): The blank, standard and sample were prepared. The blank contains Biuret reagents 1000 µl, standard was comprised of protein standard 10 µl mixed with Biuret reagents 1000 µl, and in the last cuvette serum 10 µl and Biuret reagents 1000 µl. The contents of cuvettes were mixed for 10 minutes and absorbance were measured at 546 nm

Cholesterol : The blank, standard and sample were prepared. The blank contains prepared reagent 1000 µl, standard was comprised of cholesterol standard 10 µl mixed with reagents 1000 µl, and in the last cuvette serum 10 µl and reagents 1000 µl. The contents of cuvettes were mixed for 10 minute and absorbance were measured at 510 nm.

Albumin (BCG Method): The blank, standard and sample were prepared. The blank contains BCG reagent 1500 µl, standard was comprised of albumin standard 10 µl mixed with BCG reagent 1500 µl, and in the last cuvette serum 10 µl and BCG reagent 1500 µl. The contents of cuvettes were mixed for 5 minutes and absorbance were measured at 628 nm

Bilirubin (Total): Two cuvettes were selected i.e. sample and blank, the only difference in these two cuvettes was R2 1 drop, dropped in sample. For sample R2 1 drop, R1 0.2 ml, R3 1.0 ml and serum 0.2 ml were mixed and allowed to stand for 15 minutes at room temperature. For blank R1 0.2 ml, R3 1.0 ml and serum 0.2 ml were mixed and allowed to stand for 15 minutes at room temperature. R4 1.0 ml was mixed to sample and blank and allowed to stand for 10 minutes at room temperature and absorbance was measured at 578 nm.

2.4 Precision of the method

All the methods of analysis were first checked for reproducibility and precision. The reproducibility of the method was checked by measuring the values for five replicates from human serum sample. Precision of the method for the analysis of the tests was determined by measuring the mean concentration of these parameters of five replicates of the same sample of human serum. The variation among the results were determined as $\delta/M \times 100 = \%RSD$, where δ is the standard deviation, M is mean and %RSD is the relativestandard deviation

3. Results and Discussion

The study mainly focused on heavily traffic-loaded area where public had more chances for exposure to the polluted air. The amounts of CO and NO_2 in selected area were measured by gas detectors are given in Table 1. These pollutants were monitored in the

air from 8.00 a.m. to 4.0 p.m. taking an interval of one hour duration and at a distance of 10 feet from the roadside. On the basis of levels of CO and NO_2, various locations are classified as polluted and non-polluted. The mean, standard deviation and % RSD for total protein, cholesterol, albumin and bilirubin are given in Table 2.

Age

The mean (± SD) age of the volunteers from polluted and non-polluted areas were 26.63 (± 7.24) and 29.37 (± 9.17) years, respectively. In polluted area, the ages of smokers were 27.81±7.89 years and of non-smokers were 25.39± 6.34 years. The ages of smokers and non-smokers were 30.00 (± 6.48) 28.88 (± 10.91) years in non-polluted areas. Similarly, the ages of the smokers from polluted area and non-polluted areas were 27.81± 7.89 and 30.00±6.48 years, respectively, and the differences were significant. While the ages of the non-smokers from polluted area and non-polluted areas were 25.39±6.34 and 28.88±10.91 years, respectively. In short, various biochemical parameters may not be affected by age difference.

Length of stay

The length of stay was defined as the permanent stay of volunteer in that particular area for at least 1 year. It is one of the important parameter. The mean±SD length of stay of the volunteers from polluted and non-polluted areas was 8.76±7.43 and 14.3 11.2 years, respectively. Those volunteers with the history of jaundice, tuberculosis, liver or other any serious disease in past and their period of recovery was less than 5 years and those using antibiotics or other drugs for the last 4 weeks were excluded from the study. The length of stay of smokers in polluted area was 10.28±8.53 years and of non-smokers 7.17 (± 5.74) years. The length of stay of the smokers and non-smokers in non-polluted areas was 12.50±12.35 and 15.76±10.21 years while the stay of the smokers between polluted area and non-polluted areas was10.28±8.53 years and 12.50±12.35 years, respectively. Similarly, the lengths of stay of the non-smokers from polluted area and non-polluted area was 7.17±5.75 years and 15.76±10.2) years, respectively.

Cigarettes per day (cig/day) and period of smoking

The mean (± SD) cigarettes per day of the smokers from polluted area were 10.58 (± 9.33) cig/day and from non-polluted area were 8.50 (± 6.34) cig/day. The period of smoking of the smokers from polluted area were 8.85±6.49 years and from non-polluted area were 8.92±6.50 years. It may be pointed out that use of “Naswar” is another common form of tobacco exposure especially in the studied population, but it has not been taken into account esp. in non-smokers. Certainly, use of Naswaar would also influence our results.

Total protein

Total Protein level (g/dl) in blood serum samples of human volunteers from polluted & non-polluted areas are presented in Table 3. The values of total protein in polluted area smokers (n=48) were 7.0±1.07 g/dl and, range 6.6-8.3, and non-smokers (n=46) were 7.03±1.18 g/dl. The contents of total protein in non-polluted area smokers (n=26) were 7.36± .02 and non-smokers (n=33) were 6.82±0.96 g/dl. The values of total protein in smokers from polluted area and non-polluted area were 7.0±1.07 and 7.36±1.02 g/dl. The

difference of the total protein in polluted and non-polluted smokers was not significant (P>0.05). The concentrations of total protein in non-smokers from polluted area and non-polluted area were 7.04±1.18 and 6.82±0.96 g/dl, respectively. This study shows the effect from smoking prominently on the level of total protein. Craig studied the effect of compounds associated with cigarette smoking on the secretion of lipoprotein lipid by HepG2 cells, according to his findings cigarette smoking is associated with significance alteration in serum levels of lipids and lipoproteins (10).

Cholesterol

Table 4 shows the cholesterol level (mg/dl) in blood serum samples of human volunteers from polluted & non-polluted areas. The precision of the method was determined by measuring the cholesterol level in dilution of human serum sample with saline. The cholesterol level in polluted area (n=94) of the volunteers was 163.1±41.2 and in non-polluted area (n=59) were 171.6± 34.4 mg/dl. The values of cholesterol in polluted area smokers (n=48) were 162.2± 41.2 and, range 150-240 mg/dl, and non-smokers (n=46) were 164.0 (± 41.6) mg/dl. The contents of cholesterol in non-polluted area smokers (n=26) were 176.3±35.6 and non-smokers (n=33) were 167.9± 33.5 mg/dl. The concentrations of cholesterol in smokers from polluted area and non-polluted area were 162.2± 41.2 mg/dl and 176.3± 35.6 mg/dl. The contents of cholesterol in non-smokers from polluted area and non-polluted area were 164.0±41.6 and 167.9±33.5 mg/dl, respectively. This study shows that pollution and smoking both have no significant effect on the level of cholesterol.

Albumin

Table 5 shows the serum albumin level (g/dl) in blood serum samples of human volunteers from polluted & non-polluted areas. The average serum albumin level in polluted area (n=94) of the volunteers was 4.206± 0.587 and non-polluted area (n=59) was 4.74±0.48 g/dl. The values of serum albumin in polluted area smokers (n=48) were 4.18 (± 0.65) g/dl and, range 3.5-5.0 g/dl, and non-smokers (n=46) were 4.23±0.52 g/dl. The difference was not significant (P>0.05). The concentrations of serum albumin in non-polluted area between smokers (n=26) and non-smokers (n=33) were 4.73±0.56 and 4.74±0.42 g/dl. The values of serum albumin in smokers between polluted area and non-polluted area were 4.18±0.65 and 4.73±0.56 g/dl. The contents of serum albumin in non-smokers from polluted area and non-polluted area were 4.23±0.52 and 4.74±0.42 g/dl, respectively. Hunter and co-workers studied the effect of smoking and abstention from smoking on fibrinogen synthesis in humans, according to there studies they showed that plasma albumin concentration were lower in smokers than in non-smokers (11).

Bilirubin

The bilirubin level (mg/dl) in blood serum samples of human volunteers from polluted & non-polluted areas are given in Table 7. The value of serum bilirubin level in polluted area (n=94) of the volunteers was 0.41±0.187 and non-polluted area (n=59) was 0.54±0.255 mg/dl. The values of bilirubin in polluted area smokers (n=48) were 0.442±0.217 mg/dl, range up to 1 mg/dl, and non-smokers (n=46) were 0.378 (± 0.146) mg/dl. The contents of bilirub in non-polluted area smokers (n=26) were 0.57±0.164 and

non-smokers (n=33) were 0.512±0.309 mg/dl. The concentrations of bilirubin in smokers from polluted area and non-polluted area were 0.442± 0.217 and 0.57±0.164 mg/dl. The values of bilirubin in non-smokers from polluted area and non-polluted area were 0.378±0.146 and 0.512± 0.309 mg/dl, respectively. Watanabe and co-researchers studied the effect of cigarette smoking on lipid peroxidation and liver function tests in rats, according to them the total bilirubin values were significantly higher in smoke exposure groups than those in the control group (12). This study shows that pollution has a significant effect on the level of bilirubin.

Conclusion

The present study was focused to correlate the impact of air pollution on various liver biochemical parameters. Smoking has prominent effect on the level of total protein. It has been found that pollution has an effect on serum albumin. Clear difference was observed in smokers and non-smokers living in polluted and non-polluted area. The level of serum albumin was higher in smoker living in non-polluted area compared to the polluted area. Further it shows pollution has effect on the level of bilirubin. The level of bilirubin was higher in volunteers living in non-polluted area than polluted area.

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Table 1. Level of CO and NO_2 (8 hrs average) in Peshawar areas.

S.No	Location	CO (ppm)	NO_2 (ppm)
1	Khyber Bazar	16.0	1.6
2	New Bus Stand	14.8	1.4
3	Ramdas Chowk	14.5	1.0
4	Hayatabad	8.0	0.02
5	Harichand	4.0	0.01
	Permissible Levels	9.0	0.05

Table 2. Statistical analysis of various parameters

Parameter	Mean	Std. Dev.	%RSD
Total protein (g/dl)	8.04	0.11	1.36
Cholesterol (mg/dl)	190.6	2.07	1.08
Albumin (g/dl)	4.16	0.20	4.80
Bilirubin (mg/dl)	0.40	0.01	0.20

Table 3. Total Protein level (g/dl) in blood serum samples of human volunteers.

S.No	Area	N	Min	Max	Mean	St. Dev	%RSD
1	Polluted Smoker	48	5.20	9.80	7.00	1.07	15.28
2	Polluted Nonsmoker	46	4.50	10.40	7.03	1.18	16.78
3	Non-polluted Smoker	26	5.50	9.40	7.36	1.02	13.86
4	Non-polluted Non-smoker	33	5.10	9.0	6.82	0.957	14.03

Table 4. Cholesterol level (mg/dl) in blood serum samples of human volunteers.

S.No	Area	N	Min	Max	Mean	St. Dev	%RSD
1	Polluted Smoker	48	87.0	284.0	162.19	41.2	25.40
2	Polluted Nonsmoker	46	82.0	289.0	164.04	41.6	25.36
3	Non-polluted Smoker	26	123.0	252.0	176.31	45.6	25.86
4	Non-polluted Non-smoker	33	90.00	223.0	167.88	33.9	20.19

Table 5. Serum Albumin level (g/dl) in blood serum samples of human volunteers.

S.No	Area	N	Min	Max	Mean	St. Dev	%RSD
1	Polluted Smoker	48	3.00	5.60	4.18	0.725	17.34
2	Polluted Nonsmoker	46	3.30	5.50	4.23	0.521	12.32
3	Non-polluted Smoker	26	4.00	6.00	4.73	0.557	11.78
4	Non-polluted Non-smoker	33	3.90	6.10	4.74	0.421	8.88

Table 6. Bilirubin level (mg/dl) in blood serum samples of human volunteers.

S.No	Area	N	Min	Max	Mean	St. Dev	%RSD
1	Polluted Smoker	48	0.10	1.10	0.44	0.21	47.73
2	Polluted Nonsmoker	46	0.10	0.80	0.37	0.14	37.84
3	Non-polluted Smoker	26	0.20	0.90	0.56	0.16	28.57
4	Non-polluted Non-smoker	33	0.10	1.60	0.51	0.30	58.82