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# Determination of Trace Amounts of Iron, Copper, Nickel, Cadmium and Lead in Human Blood by Atomic Absorption Spectrometry

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**Abstract:** The trace amounts of Fe, Ni, Cu, Cd and Pb were determined in 73 selected human blood samples collected from seven groups of general population including normal and diseased persons. Atomic absorption spectrometric technique was used for these measurements. Iron and Cu were found in variable amounts in almost all samples studied. Among toxic metals, Cd was found negligible in all groups whereas, Pb was present in relatively high concentration. Elevated level of Ni was noted in few samples only. The correlation of these metals to age of the group members was calculated statistically. Possible sources of blood contamination by the toxic elements, their adverse impacts on human health and the diseases caused by the excess or deficiency of essential trace elements were also considered.

**Key words:** Human blood, essential, toxic, trace metals, deprotienization, environmental contamination

## Introduction

A human body is basically composed of macro molecules such as proteins, carbohydrates, fats and nucleic acids. These molecules result from chemical combination of C, H, N, O, and P as the major elements (Khurshid *et al.*, 1984). Besides these constituents, human body contains a number of other elements and complex compounds which influence many normal body functions. These elements are assimilated through food, water and the environment (Wahid *et al.*, 2001). WHO (1980) has recommended the limits of trace metals for normal functions of a body and balance of these constituents is disturbed due to various types of pollution. Lower or higher concentration of these elements in body adversely affect the normal biological functions. Metals such as Fe, Ni, and Cu are essential for human body but chronic metabolic disturbances may occur due to the deficiency or excess of these metals (Mertz, 1981). According to Nielsen (1982), non-essential elements such as Pb and Cd are considered to be toxic and their presence in the body can cause profound biochemical and neurological changes in the body even at ultra trace level. Primary sources of metal pollution are various metallurgical processes such as metal extraction, purification, mining, smelting and refining. Among other sources, fossil fuels such as coal and oil release the metallic particles whereas, the most diverse source of pollution is the production of industrial products containing metals in various forms (Talib *et al.*, 1990). These metallic pollutants follow many pathways to enter into the environment. In case of human body, main routes of exposure to these metals are absorption through skin, direct inhalation and gastrointestinal absorption (Talib *et al.*, 1990). After absorption, these metals enters into the blood stream either from the pulmonary or gastrointestinal tract and then transported around the body. According to Berman (1980) continuous exposure to these elements results in accumulation in the body and elevate their level in tissues and fluids. Among body parts, blood is considered to be the most reliable parameter for the assessment of exposure of metal pollutant in general population (McAughey *et al.*, 1987; Hassler *et al.*, 1983) because these metals reach different parts of body through blood circulation and cause serious health problems.

Islamabad being the capital of Pakistan is a blend of varied cultures and become a real global village. Besides the fallout of tetra-alkyl lead from gasoline, randomly increasing transportation and production of variety of wastes, there are more than 170 industrial units located in its various sectors, which are the major sources of environmental pollution. Despite these facts, there is no any environmental monitoring or data available regarding the level of toxic metals in human blood. Keeping this in view, present study was undertaken to determine and establish a baseline level of trace metals in human blood and their correlation to age and health problems of the people.

## Materials and Methods

To collect the information and blood samples of patients suffering from different diseases, a comprehensive survey of various health centers of Islamabad city was conducted from September, 1998 to February, 1999. For convenience, population was divided into seven different groups to assess the degree of exposure of selected metals. The groups were based on gender difference and disease. These were normal (healthy) and exposed (worker) male and female groups of population. The blood sampling was made properly among the selected groups of patients namely diabetes mellitus (DM), asthma-respiratory tract infection (ARTI), enteric fever (EF), hepatitis (HT), arthritis (Art) and Gynae (Gyn) from various health centers of Islamabad. Analyses were carried out in well equipped IAEA standard laboratory of Pakistan Institute of Nuclear Science & Technology (PINSTECH) using atomic absorption technique.

**Blood sampling:** Blood samples (5 ml each) of seven selected groups (73 samples) of population were collected by venous puncture from Pakistan Institute of Medical Sciences (PIMS), Polyclinic and, Capital hospitals and various residential sectors of Islamabad. The puncture site was cleaned to remove any expected contamination before sampling. Separate and disposable sterilized plastic syringes were used for blood collection.

**Deprotienizing and preparation of samples:** Among deprotienizing agents, nitric acid being an excellent reagent was used for deprotienization of blood samples (Subramanian, 1987). Deprotienized blood samples were transferred to pre-cleaned centrifuge tubes and allowed for complete clotting. Blood serum was separated as supernatant after centrifugation and collected in 10 ml pre-cleaned measuring flasks containing a 1:4 mixture of 2 ml of 1 M $HNO_3$ and HCl. Final volume of the samples was made with de-ionized-water before analyses.

**Reagents and solutions:** Analytical reagent grade HCl and $HNO_3$ were procured from E. Merck, Germany. Certified standard stock solutions of Fe, Cu, Ni, Cd and Pb were obtained from BDH, England for calibration purpose. Fresh standard solutions were prepared from stock solutions on each day of analysis by dilution with 5% $HNO_3$ solution in distilled water. Blank solutions were treated and prepared exactly in the same way as the samples. All glassware and apparatus used were cleaned by soaking in 5% $HNO_3$ followed by rinses with distilled water before use. All working solutions were also prepared in de-ionized distilled water.

**Instrumentation:** On the basis of high sensitivity and simplicity (Gurdeep *et al.*, 1990; Subramanian, 1987), a double beam atomic absorption spectrophotometer Model 180-80 Zeeman Hitachi with automatic background correction was used for these determination. Centrifuge Machine Model D-7209 Gosheim F.R Germany, was employed for blood centrifugation.

## Results and Discussion

Despite the fact that Islamabad is a modern, and well planned newly populated city of Pakistan, there are various environmental degradation factors such as rapid urbanization, industrialization and ever increasing transportation which continuously pollute its environment. Present attempt was made in the context to study the level of exposure of human being to metallic pollutants.

The concentration of iron in seven selected groups of population of both sexes living in and around the city have been determined (Tables 1-4). Mean iron concentration for normal group of population was found to be 6.8 ppm. All except diabetes mellitus patients (56.42 ppm) have shown low mean concentration of iron ranging from 0.89 to 6.8 ppm. The correlation(r-value) for Fe accumulation in the body with age was calculated to be significant in all groups except HT and ARTI patients, where it was noted to be negative (-0.023 and –0.118). Among essential trace metals, iron plays an important role in oxygen transport and cellular respiration. Vasudevan *et al.* (1996) has reported 10-20 mg as daily body requirements of iron out of which only 1-2 mg is absorbed. However, iron deficiency causes many serious diseases such as hemolytic anemia, palpitation and reduction in physical activities (Mushtaq, 1988) whereas, excessive intake of ferrous compounds are associated with liver and kidney damage, hepatic enlargement, diabetes and myocardial diseases (Khurshid *et al.*, 1984; Mushtaq, 1988). The data agreed well for DM and some other groups of patients under treatment in various health centres.

Mean concentration of copper was found to be 1.4, 1.95,1.28, 2.68, 2.53, 9.26 and 2.58 ppm for normal, Dm, ARTI, EF, HT, Art, and Gyn patients respectively (Tables 1-4). Copper contents were found many folds higher in the blood of arthritis group and in few EF and HT patients. Comparatively low concentration of Cu was noted for normal persons. The value of 'r' for various groups

Table 1: Determination of iron, copper, nickel, lead, and cadmium in blood serum of normal or healthy persons (concentration in ppm)

| Sr. No. | Sex/Age (years) | Fe | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| 1. | M/35 | 4.7 | 1.0 | 0.5 | 3.2 | 0.05 |
| 2. | M/50 | 8.5 | 1.7 | 1.3 | 3.7 | 0.02 |
| 3. | M/21 | 7.3 | 0.65 | 0.8 | 2.5 | -- |
| 4. | M/33 | 3.7 | 0.15 | -- | 3.2 | -- |
| 5. | M/60 | 29.7 | 1.70 | -- | 4.7 | -- |
| 6. | M/55 | 10.2 | -- | -- | 1.5 | -- |
| 7. | M/48 | 2.5 | 1.8 | 0.6 | 1.4 | -- |
| 8. | M/27 | 5.1 | 2.6 | 1.7 | -- | -- |
| 9. | M/38 | 11.8 | 1.25 | -- | 2.8 | -- |
| 10. | FM/22 | 2.7 | 0.35 | -- | -- | -- |
| 11. | FM/27 | 7.15 | 2.2 | 1.35 | 2.1 | -- |
| 12. | FM/40 | 2.3 | 1.3 | 0.56 | 2.7 | -- |
| 13. | FM/44 | 3.9 | 0.9 | -- | 4.2 | -- |
| 14. | FM/49 | 1.8 | 2.8 | 0.3 | 3.5 | 0.06 |
| 15. | FM/53 | 4.1 | 2.0 | -- | 4.3 | 0.05 |
| 16. | FM/45 | 2.7 | 1.2 | -- | 2.6 | -- |
| 17. | FM/24 | 5.2 | 1.6 | -- | 1.3 | -- |
| 18. | FM/30 | 9.3 | 0.07 | -- | -- | -- |
| Mean. | -- | 6.8 | 1.4 | 0.88 | 2.87 | 0.04 |
| (r)[c] | -- | 0.348 | 0.145 | -- | 0.447 | -- |

M, FM for Male & Female patients
C, Correlation with age.

Table 2: Determination of iron, nickel, copper, cadmium and lead concentration (ppm) in blood of Diabetes mellitus and asthma-respiratory tract infection group of patients.

| Sr. No. | Sex/Age (Years) | Fe | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| 1(DM)[a] | M/60 | 21.79 | 2.75 | 7.0 | 7.60 | 0.18 |
| 2. | M/52 | 44.90 | 3.25 | -- | 5.65 | 0.01 |
| 3. | M/54 | 0.78 | 2.18 | -- | 3.88 | -- |
| 4. | M/50 | 0.38 | 2.50 | -- | 2.00 | 0.25 |
| 5. | M37 | 0.15 | 1.25 | -- | 1.60 | -- |
| 6. | M/54 | 430.57 | 2.75 | 4.25 | 3.25 | 0.01 |
| 7. | M/18 | 8.28 | 0.33 | -- | -- | 0.01 |
| 8. | FM/43 | 0.28 | 1.25 | -- | 2.10 | -- |
| 9. | FM/30 | 0.50 | 2.00 | -- | 3.00 | -- |
| 10. | FM/33 | 0.35 | 1.25 | -- | 1.10 | -- |
| Mean | -- | 56.42 | 1.95 | 5.62 | 3.35 | 0.09 |
| (r)[c] | -- | 0.327 | 0.84 | -- | 0.709 | -- |
| 1(ARTI)[b] | M/35 | 14.13 | 1.75 | -- | 1.25 | 0.11 |
| 2. | M/76 | 1.55 | 0.50 | -- | 1.28 | -- |
| 3. | M/31 | 1.63 | 0.75 | -- | 2.80 | 0.03 |
| 4. | M/45 | 10.40 | 1.75 | -- | 1.90 | -- |
| 5. | M/44 | 0.65 | 1.25 | -- | 3.40. | -- |
| 6. | FM/30 | 0.50 | 2.25 | -- | 2.38 | 0.02 |
| 7. | FM/32 | 3.18 | 1.25 | -- | -- | -- |
| 8. | FM/40 | 2.7 | 1.45 | -- | 2.63 | 0.02 |
| 9. | FM/50 | 4.2 | 0.65 | -- | 1.12 | 0.05 |
| Mean. | -- | 4.80 | 1.28 | -- | 2.09 | 0.04 |
| (r)[c] | -- | -0.118 | -0.59 | -- | -0.449 | -- |

[a]Diabetes mellitus [b]Asthma and respiratory tract infection
[c]Correlation with age

Table 3: Determination of iron, nickel, copper, cadmium and lead, in blood of patients affected by hepatitis and enteric fever (concentration in ppm)

| Sr. No. | Sex/Age (Years) | Fe | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| 1. EF[a] | M/14 | 4.10 | 1.50 | -- | 3.25 | -- |
| 2. | M/20 | 4.62 | 11.00 | -- | 2.45 | -- |
| 3. | M/28 | 1.25 | 3.25 | -- | -- | -- |
| 4. | M/10 | 0.75 | 1.25 | -- | 8.40 | -- |
| 5. | FM/29 | 4.13 | 2.50 | -- | -- | -- |
| 6. | FM/11 | 1. 35 | 1.25 | -- | 2.80 | -- |
| 7. | FM/13 | 2.5 | 1.43 | -- | -- | -- |
| 8. | FM/15 | 1.8 | 1.50 | -- | 3.33 | -- |
| 9. | FM/14 | 0.28 | 0.50 | -- | -- | -- |
| Mean | -- | 2.30 | 2.68 | -- | 4.04 | -- |
| (r)[c] | -- | 0.383 | 0.359 | -- | -0.618 | -- |
| 1 HT[b] | M/22 | 8.05 | 9.3 | 32.75 | 1.65 | -- |
| 2. | M/39 | 5.3 | 3.25 | -- | -- | -- |
| 3. | M/14 | 2.10 | 1.00 | -- | -- | -- |
| 4. | M/50 | 0.33 | 1.5 | -- | -- | 0.53 |
| 5. | M/21 | 2.13 | 2.25 | 88.50 | 3.53 | 0.13 |
| 6. | FM/15 | 1.4 | 1.25 | -- | -- | -- |
| 7. | FM/52 | 2.57 | 0.73 | 0.50 | 2.23 | -- |
| 8. | FM/45 | 3.27 | 1.00 | -- | -- | -- |
| Mean. | -- | 3.14 | 2.53 | 40.58 | 2.47 | 0.33 |
| (r)[c] | -- | -0.023 | 0.326 | -- | - 0.243 | -- |

[a]Enteric fever [b]Hepatitis [c]Correlation with age

Table 4: Determination of iron, nickel, copper, cadmium and lead concentration (ppm) in blood serum of gynae and arthritis patients

| Sr. No. | Sex/Age (Years) | Fe | Cu | Ni | Pb | Cd |
|---|---|---|---|---|---|---|
| 1.Art.[a] | M/28 | 2.20 | 2.50 | 64.00 | 2.13 | -- |
| 2. | M/13 | 1.35 | 1.25 | -- | 1.00 | -- |
| 3. | M/23 | 0.15 | 2.25 | -- | -- | -- |
| 4. | FM/30 | 1.58 | 3.25 | 19.25 | 8.10 | 0.07 |
| 5. | FM/45 | 2.48 | 59.00 | 16.00 | 7.10 | -- |
| 6. | M/42 | 0.65 | 1.25 | -- | -- | -- |
| 7. | FM/49 | 2.1 | 1.9 | 3.7 | 1.55 | -- |
| 8. | FM/55 | 1.3 | 2.7 | 1.6 | 2.3 | -- |
| Mean | -- | 1.47 | 9.26 | 20.91 | 3.69 | 0.07 |
| (r)[c] | -- | 0.282 | 0.269 | -- | 0.100 | -- |
| Gyn[b] | FM/25 | 0.65 | 2.50 | 2.4 | 2.58 | -- |
| 2. | FM/41 | 0.33 | 4.50 | -- | -- | |
| 3. | FM/30 | 4.67 | 2.0 | -- | -- | -- |
| 4. | FM/33 | 0.3 | 3.25 | 0.5 | 1.00 | -- |
| 5. | FM/19 | 0.13 | 4.00 | 1.2 | -- | 0.06 |
| 6. | FM/25 | 0.21 | 4.50 | -- | -- | -- |
| 7. | FM/23 | 0.38 | 1.25 | -- | 1.30 | -- |
| 8. | FM/34 | 0.25 | 1.55 | -- | 1.2 | -- |
| 9. | FM/35 | 0.77 | 0.45 | -- | 1.7 | -- |
| 10. | FM/42 | 1.2 | 1.8 | -- | 1.3 | -- |
| Mean | -- | 0.89 | 2.58 | 1.36 | 1.51 | 0.06 |
| (r)[c] | -- | 0.097 | -0.168 | -- | -0.454 | -- |

[a]Arthritis [b]Gynae [c]Correlation with age

Table 5: Comparison of toxic trace metals concentration found in blood samples of two Cities [a,b]

| Metals | Metal Conc., Age & r-value | Normal (M) | Exposed (M) | Normal (FM) | Exposed (FM) |
|---|---|---|---|---|---|
| (Reported work)[a] | Age range (years) | 1- 78 | 21- 61 | 20 - 60 | 12 - 75 |
| Cadmium | Conc. range (µg ml$^{-1}$) | 6.5-64.23 | 35.29 -80.10 | 10.22- 72.75 | 16.00- 80.20 |
| --- | r- value[c] | 0.79 | 0.91 | 0.88 | 0.78 |
| Lead | Conc. range (µg ml$^{-1}$) | 6.0 -27.1 | 22.2- 54.3 | 2- 26.2 | 23.4- 65.6 |
| --- | r- value[c] | - 0.09 | 0.67 | 0.83 | 0.63 |
| (Present work)[b] | Age range (years) | 21 - 60 | 10 - 76 | 22 - 53 | 11- 55 |
| Cadmium | Conc. range (µg ml$^{-1}$) | 0.02- 0.05 | 0.01-0.53 | 0.04-0.06 | 0.01-0.07 |
| --- | r- value[c] | - 1.0 | 0.318 | - 1.0 | - 0.274 |
| Lead | Conc. range (µg ml$^{-1}$) | 1.4 - 4.7 | 1.00-8.40 | 1.3 - 4.2 | 1.10- 8.10 |
| --- | r- value[c] | 0.128 | 0.153 | 0.250 | 0.110 |

[a]Lahore (Talib *et al.*, 1990) [b]Islamabad (Present work, 2002) [c]Correlation with age

have been calculated and found to be positive for all with exception of ARTI and Gyn patients. The possible sources of Cu-elevation in blood of some patients may be the exposure of individuals to Cu-polluted environment such as corrosion of copper alloys, metal plating, industrial and domestic wastes, use of roof materials, cooking utensils, coins, pigments, insecticides, fungicides and dental materials (Berman, 1980). Moreover, according to Hinks (1983) women on oral contraceptives containing estrogen have an elevated level of Cu in their blood. The functions of Cu in human body was reported to be complementary to iron and its deficiency cause reduction in enzyme activity, hypochromic anemia and myocardial infarction (Vasudevan *et al.*, 1996) whereas, excessive Cu intake may causes many diseases such as necrotic hepatitis, thalassemia, cirrhosis, tuberculosis, carcinoma, haemolysis and Wilson's disease (Vasudevan *et al.*, 1996; Mushtaq, 1988).

The mean concentration of Ni was found very low (< 1 ppm) in only few samples of normal group of population ranging from 0.3 to 1.7 ppm (Table 1) whereas, its complete absence was recorded for ARTI and EF patients. Elevated level of Ni was noted in few HT, (32.75 ppm, 88.5 ppm) and Art (64.00 ppm, 21.0 ppm) patients (Tables 3-4). Mancean *et al.* (2000) has recently reported that consumption of fossil fuel, smoking and presence of non-ferrous metallurgical dust release metal pollutants in atmosphere. Appropriate amount of Ni in human body plays an important role in regulating prolactin and stabilization of RNA and DNA structures (Khurshid *et al.*, 1984). Excessive intake of Ni (2-7 mg/day) accumulate in lungs and becomes toxic leading to sever allergic reactions, bronchial asthma, dermatitis, eczema and myocardial infarction (Murti *et al.*, 1989). Many abnormalities in plasma cholesterol level, glycogen and mitochondrial morphology have been reported during nickel deficiency (Khurshid *et al.*, 1984).

The presence of cadmium was recorded very low (< 1 ppm) in few samples (Tables 1-4). This may be due to the fact that Cd is not present in human body at birth (Khurshid *et al.*, 1984) and its other possible sources such as pork, crab and lobster are not in practice in our country (Talib *et al.*, 1990). These findings indicated Cd free environment. Unfortunately, Cd is one of the most toxic element and its presence can cause serious biochemical and neurological changes in human body even at ultra trace level (Nielsen, 1982). The concentration of Cd in blood of general population of various countries have been reported by Talib *et al.* (1990). These were 16.14 µg ml$^{-1}$ (China), 2.1 µg ml$^{-1}$ (India), 5.54 µg ml$^{-1}$ (Japan), 1.14 µg ml$^{-1}$ (UK) and 2.14 µg ml$^{-1}$ for USA. Moreno *et al.* (1999) have recently reported the level of Cd and Pb in whole blood samples in a group of 82 people (Spain). These were 0.98 µg ml$^{-1}$ and 46.7 µg ml$^{-1}$ for Cd and Pb respectively. The comparison of these and other data clearly indicated that overall trace metal concentration in blood of the people of the area studied is low whereas, environment is Cd free at the moment.

The lead concentration in various blood samples indicated that the averages of Pb contents for normal, Dm, ARTI, EF, HT, Art, and Gyn groups of population were found to be 2.87, 3.35, 2.09, 4.04, 2.47, 3.69, and 1.51 respectively. High concentration of Pb was noted in few samples only. The correlation of Pb with age of the people has been found significant with the exception of EF, HT, ARTI and Gyn where it was recorded negative (Tables 1-4). Lead is a common global air pollutant and its toxicity affects directly the activities of different enzymes working in body. Symptoms of acute lead poisoning are tiredness, anemia, uric acid elevation, discomfort and irritability (Khurshid *et al.*, 1984). The concentration of Pb in human blood of general population of some developed countries have been reported (Talib *et al.*, 1990) to be 15.0 µg ml$^{-1}$ (China), 11.6 µg ml$^{-1}$ (India), 4.0 µg ml$^{-1}$ (Japan), 11.9 µg ml$^{-1}$ (UK) and 6.1 µg ml$^{-1}$ for USA.

The comparison of toxicity level of two cities, Lahore and Islamabad has also been given (Table 5). The concentration range of Cd in blood of normal males and females was reported to be 6.5-64.23 µg ml$^{-1}$ and 10.22-72.75 µg ml$^{-1}$ respectively whereas, in present studies it was found very low (0.02-0.05 µg ml$^{-1}$ and 0.04-0.06 µg ml$^{-1}$). Similarly, the concentration of Pb was reported to be 6.0- 27.1 µg ml$^{-1}$ and 2.0 - 26.2 for both normal sexes. Cd and Pb contents were very high in the blood of both exposed sexes. These findings agreed well with the fact that environment of an industrial and highly populated city is more polluted than the environment of a clean, well planned, less industrial and less populated city. These results have also indicated that accumulation of Cd in human body with age is significant in the reported data (Talib *et al.*, 1990) whereas, in present studies it has not shown positive relation with age. This may be due to the fact that Cd concentration is negligible in the analyzed samples. Only few samples contain Cd in very low concentration (< 1 ppm) however, metal to age relation was found significant (0.91 , 0.318) for exposed males in both reports. Regarding the accumulation of Pb with age, present results agreed well with the reported data except normal male group of population. The overall comparison of data showed very low level of blood contamination in the results reported herein.

This communication presents the baseline level of trace metals in human blood serum of selected population groups of Islamabad city. Iron, copper and lead were found in almost all groups, however, high concentration of these metals was noted in some samples only. Most of the samples were found free of Ni and Cd contents. The correlation of trace metals concentration with the age of various groups has been calculated and found to be significant for some groups of population. It was concluded that the environment of selected city is well below the toxic range at the moment.

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