Correlation of arsenic level in drinking water and hair of male respondents of district Sheikhupura
Moneeza Abbas, Kausar Jamal Cheema, Rabia Shehzadi

Abstract
Objective: To find out the correlation between arsenic concentration in drinking water and biological samples of young male respondents.

Method: The cross-sectional study was conducted at Lahore College for Women University, Lahore, Pakistan, from March 2013 to February 2015, and comprised biological samples i.e. hair of young males aged 15-25 years from Sheikhupura district of the Punjab province. The study questionnaire was designed to assess the general health status of the subjects as well. Drinking water samples were collected from the houses of those who volunteered to fill up the questionnaire and gave hair samples for the study. Acid digestion method was used to pre-treat the water and hair samples for the analysis of arsenic content. Atomic absorption spectrophotometer method was used to measure arsenic concentration. SPSS 13 was used for data analysis.

Results: There were 100 subjects and as many water samples. Overall, 46(46%) respondents had a range of health problems, including acne, skin lesions, allergies and respiratory problems. The average arsenic concentration was higher in water samples of tehsil Sheikhupura (67.11±3.8µg/L) and Sharaqpur (61.65±3.3µg/L) than Muridke (41.7±1.5 µg/L), Ferozwala (40.79±1.3 µg/L) and Safdarabad (29.7±0.5 µg/L) (p<0.05). Positive correlation was established between arsenic in drinking water and hair samples with respect to area and age (p<0.05).

Conclusion: The presence of arsenic in drinking water was found to be likely affecting general metabolism and its accumulation in biological tissues.

Keywords: Arsenic, Drinking water, Hair, Male respondents. (JPMA 69: 499; 2019)

Introduction
Arsenic (As) exposure is a major health issue affecting more than 300 million people worldwide. Cancers of bladder, skin, kidney, intestine and prostate have been known to be caused by elevated As levels in drinking water.1 For the general population, As-contaminated drinking water is the major exposure source and more destructive than As-contaminated food because the bioavailability of As from drinking water is more than that from vegetables and grains.2 The As contaminations in water resources can be due to multiple factors. Increased industrial activity, rapid urbanisation and agricultural requirement of chemicals and fertilisers have led to water pollution.3

The amount of As or its metabolites in biological materials such as hair, blood, nails and urine are used as biomarkers of As association.4 After absorption, As accumulates in biological samples such as in hair and nails, and it is thought to be involved in the binding of As to the sulphhydryl groups in keratin. Hair is the most appropriate As bio-indicator compared to other biological tissues because it is not a dynamic tissue and remains considerably invariant.5

There is a significant positive correlation between As concentration in drinking water and biological samples like hair, nail, blood etc. A study6 on blood, nails and urine from areas with As-contaminated drinking water revealed a strong relationship between As level in drinking water and its accumulation in the biological samples. In Pakistan's Punjab province, As-contaminated water and general environmental degradation are growing concerns. Naturally occurring As and various
anthropogenic activities are adding up to the problem, and, hence, pose a serious threat to ecological and human health. The current study was planned to do quantitative determination of As in drinking water supply to highlight the point sources of As contamination with alarming risk levels. It also planned to find out As levels in the hair of young males and to explore the correlation of As levels in the samples with drinking water and the consequent health risk assessment.

**Subjects and Methods**

The cross-sectional study was conducted at Lahore College for Women University (LCWU), Lahore, Pakistan, from March 2013 to February 2015, and comprised drinking water and biological samples from Sheikhupura district of the Punjab province. After getting approval from the institutional ethics committee, samples of drinking water and hair were collected by using non-probability sampling technique. The respondents were permanent residents of five tehsils of the district and were mostly students at different institutions. Water samples were collected from the houses of the respondents who were males aged 15-25 years. Different colleges and educational institutions were visited and meetings were held to explain the purpose of the study. Written permission was obtained from institutional heads prior to conducting interviews and administering the questionnaire to the volunteers. Samples were collected only for those who filled up the consent form.

Sample size was calculated statistically by using the formula

\[
\text{Sample Size (SS)} = \frac{Z^2 \times p \times (1-p)}{C^2}
\]

Where,
- \(Z\) = confidence level e.g. 1.96 for 95%
- \(p\) = percentage picking a choice, expressed as decimal i.e. 50% (0.5)
- \(C\) = confidence interval, expressed as decimal i.e. 0.05

Now by putting values

\[
\text{Sample Size} = \frac{(1.96)(1.96)(0.5)(1-0.5)}{(0.05)(0.05)} = \frac{(3.84)(0.25)}{0.0025} = 384
\]

Hence, the calculated sample size was 384

But due to limitations in the sample size it was impossible to collect such a large number of samples and some adjustments in the formula were made as follows:

\[
\text{New Sample size (NSS)} = \frac{SS}{SS-1} + 1
\]

Where,
- \(SS\) = Sample size calculated above i.e. 384
- \(Pop\) = Population size i.e. 350 as survey done in our research

Now by putting values

\[
\text{New Sample Size} = 192
\]

Due to the age limit and single-gender specifications of our study, the sample size was further reduced. A total of 100 water and hair samples were collected which added up to 200 samples of biological and environmental media, and justified sample size requirement of 192.

For the assessment of health status, a structured questionnaire was used. All the samples were sealed and transported to the LCWU Environmental Science Laboratory for further analysis.

Water samples were pre-treated for As detection by adding 2ml of 30% hydrogen peroxide (H\(_2\)O\(_2\)) into 100ml of the sample. Then a few drops of concentrated nitric acid (HNO\(_3\)) were added. Solution was heated at 95°C till digestion was over or it was continued until the leftover volume was reduced to 50ml. After wet cooling, the solution was transferred to a clean volumetric flask, and brought the total volume to 50ml with water. Next, 5ml of this solution (from wet digestion) was taken by pipette and added to the volumetric flask, followed by addition of 1% nickel nitrate solution (1ml) and diluted to 10ml with reagent water. Hair samples were weighed (0.1g) by using a weighing balance (Sartorious TE31-DS, Germany). De-ionised water was used for washing the samples. They were further washed with methanol to remove any debris without leaching As out of them. Hair samples were then treated with 8ml concentrated HNO\(_3\) for decomposition in crucibles enclosed with the crucible lid and were placed on the hot plate. Hair digestion was carried out at temperature between 70°C and 85°C for 25 minutes and the clear solution of digested hair samples was obtained. The samples were not allowed to dry until the digestion was completed. Crucibles containing digested hair samples were cooled to ambient temperature, 1ml of 30% H\(_2\)O\(_2\) was added and the samples were again heated.
on the hot plate at the lowest setting (42°C) just until bubbling ended. Temperature was then increased to 80°C or even above to reduce the volume of the sample to 2.5ml. The remaining contents of the samples were transferred from the crucible to dried and cleaned volumetric flasks quantitatively. Final volume of samples was made using de-ionised water. Solution was filtered using Whatman filter paper of size 47mm (2 micron) and transferred to properly labelled and cleaned sample bottles, and stored in the refrigerator till analysed.8

For the Analysis of arsenic detection, both hair and drinking water samples were run on atomic absorption spectrophotometer (Solar MKII-VI Thermo Electron Corporation, UK). For statistical analysis, Minitab V 13 software was used to compute means and standard deviations (SD) for As concentrations, along with correlations between water and hair samples. SPSS 13 was used to conduct independent sample student's t-test and analysis of variance (ANOVA) later to compare means across the groups.

Results
There were 100 subjects and as many water samples. Overall, 46(46%) respondents had a range of health problems, including acne, skin lesions, allergies and respiratory problems.

The mean As concentration was higher in water samples of tehsil Sheikhupura (67.11±3.8 µg/L) and Sharaqpur (61.65± 3.3 µg/L) compared to Muridke (41.7± 1.5 µg/L), Ferozwala (40.79± 1.3 µg/L) and Safdarabad (29.7±0.5 µg/L) (p<0.05) (Figure 1).

The mean arsenic concentration in hair samples was higher in respondents of tehsil Sheikhupura (1.10±0.183 µg/g) and the lowest was in those belonging to tehsil Safdarabad (0.28±0.118 µg/g) (Figure 2).

A significant positive correlation (r =1.0) was found between average As level in the samples, confirming that As level in drinking water directly affected the As content in the hair samples (Figure 3).

Average As level was higher in hair sample of age group of 23-25 years (1.16 ±0.15 µg/g) compared to the 19-22 years group (0.60 ±0.11 µg/g) (p<0.05) (Figure 3).
years age group (1.0 ± 0.12 µg/g) and the 15-18 years age group (0.54 ± 0.1 µg/g). The mean As concentration in different age groups was significantly higher (p<0.05) than the permissible limit of 0.008-0.25 µg/g (Figure 4).

There was a slightly positive correlation between As level in drinking water samples with As content in the hair samples belonging to age groups from all tehsils (Figure 5).

**Discussion**

Like India, Bangladesh and other countries that are facing high levels of As in the drinking water, Pakistan is now also having the menace of As contamination. The present study was conducted for the determination of As concentration in drinking water supplies and hair of the male population of district Sheikhupura which is an agricultural as well as industrial district. The findings indicated that As contamination in drinking water supplies to the areas was a matter of concern. In some tehsils, the level of As in hair and water samples was above the permissible limits.

It was evidently shown that the concentration of detected As was much higher in samples from tehsil Sheikhupura and Sharaqpur. The presence of high level of As in Sheikhupura district may be due to some agricultural activities or industrial untreated effluent. According to a study, chronic As toxicity due to untreated industrial effluent affected 14 villages in India. Similar results of high As concentration in drinking water samples collected by female respondents in tehsil Sheikhupura and Sharaqpur was found by another study. One study found that ground water had become contaminated with As due to the release of untreated industrial effluent by a local factory after the production of insecticides.

The samples in the current study were collected from five types of drinking water sources. It was noted that mean±standard error of mean (SEM) As concentration was higher in hand-pump water. This may be attributed to the fact that water is drawn from a depth ranging 80 to 100 feet by the hand-pump. So water is comparatively more polluted than water supplies of the Water and Sanitation Authority (WASA) as it pumps water from 500 feet down or even more. These observations reflect on the fact that drinking water quality of district Sheikhupura is compromised.

The average As concentration in hair samples was higher in male respondents of tehsil Sheikhupura. These results complement the results of water samples analysed from various tehsils. This might be due to fact that male respondents of tehsil Sheikhupura had more intake of contaminated water compared to samples collected from other tehsils. As concentration observed in the present study was higher than reported normal range of arsenic.
(0.08-0.2 µg/g). A study\textsuperscript{11} reported similar results, concluding that As concentrations in hair were clearly higher in individuals consuming As-contaminated drinking water compared to those consuming municipal treated water with low As concentration.

Further, higher mean As level was found in higher age group (23-25 years) and lower values in the lower age groups. A direct correlation was found between age and As level. It might be due to exposure to As-contaminated water for longer period of time.\textsuperscript{12}

There was an increasing trend between As in water samples and hair of the male population of three age groups belonging to five tehsils of district Sheikhupura. The results are in accordance with an earlier study\textsuperscript{6} which concluded that biological samples (blood, nails and urine) from areas with As-polluted drinking water revealed a positive correlation between As level in drinking water and in biological samples.

Another study\textsuperscript{13} reported correlation between As level in biomarkers and daily As exposure through diet and drinking water among population living in As-contaminated areas. It was observed that in As-contaminated regions, significantly higher As concentration in hair and urine samples of the participants was strongly linked with higher As intake through diet and drinking water, while these concentrations were found to be low in people living in non-contaminated areas.

The current study provides evidence that hair is a good biomarker of As exposure that can be used for the study of such exposure in the population. The situation demands urgent and effective remedial and mitigation measures in connection with As contamination.

**Conclusion**

Average As concentration in the entire study was above the WHO permissible limit. The water quality of district Sheikhupura was not suitable, and was causing ill effects to the health of the people as well as to the area's ecological health.