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Original Article

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Hair manifestations in factory workers with arsenic exposure in Hayatabad industrial estate Peshawar, Pakistan

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ABSTRACT

BACKGROUND & OBJECTIVE: Arsenic is present both naturally and from manmade sources like factory effluents. Contamination by arsenic of the groundwater is a major source of health detriment in south-east Asian countries. The aim of this study was to identify levels of arsenic in drinking water sources and factory effluents and to analyse arsenic levels in hair samples from factory workers and their families, along with morphological changes in hair in industrial workers in Peshawar.
METHODOLOGY: Water and hair samples were collected by random sampling from a labor colony of 81 factory and non-factory household members living and working in the same industrial estate and analyzed for arsenic. Hair morphology was carried out in histomorphology laboratory at Khyber Medical University, Peshawar. The results obtained from both groups were compared with normal hair histomorphology. The data was analyzed by using SPSS version 20.
RESULTS: The arsenic level in drinking water, colony tube wells and households was not within the permissible level of WHO (10µg/L). The arsenic level in factory effluent was 68µg/l (Pakistan Government's acceptable level is 50µg/L). The difference was statistically significant p≤0.001 between the two groups, the factory workers had a relatively higher level of arsenic, ~34ppb, in their scalp hair. Hair arsenic level also came out to be <1.00µg/g. There was a wide variation in the histomorphology of the hair samples in both groups.
CONCLUSION: Elevated levels of arsenic in factory effluent demonstrate improper decontamination. The arsenic levels were within permissible levels, long term effects cannot be ruled out.
KEYWORDS: Hair, Arsenic, Histomorphology, Manifestation.

INTRODUCTION

Although heavy metals are found naturally in the earth's crust at minute levels, one of the major sources of water and soil contamination are the effluents from industries that use them in the manufacture of various products[1]. These industries are involved in the manufacture of pesticides, pharmaceuticals, textile industry, paper, glass and metal adhesives etc. The most lethal form of arsenic is arsenate (oxidative state 5) which seeps into the underground water supply[2]. Arsenic contaminated groundwater in south Asian countries is one of the largest environmental health hazards in the world including parts of Pakistan[3]. This contaminated water is used for agriculture and human consumption and is not only ingested by the human population but is also absorbed through air and skin[4]. The acceptable level of arsenic set by WHO (World Health Organization) is less than 10µgm/liter (ppb)[5].

According to WHO about 150 million people in many countries of the world are exposed to arsenic poisoning. Epidemiological studies in Pakistan have reported arsenic contamination of groundwater in Punjab higher than 10µgm/litre (10ppb), whereas the Indus plains have shown even higher levels, with 322µgm/litre (322ppb) level observed in southern Sindh [6–8]. Thus, the exposure rate to arsenic in Pakistan exceeds 60 million, making it a major health hazard. However, the Pakistan standard and quality control authority (PSQCA) set the acceptable level of arsenic in drinking water as 50µgm/litre (50ppb) due to naturally high levels in the soil[5].

According to International Agency for Research on Cancer (IARC) arsenic has been ranked as group 1 carcinogen[3,9].

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A joint FAO/WHO committee on food activities (JECFA) concluded that where the arsenic in drinking water exceeds 50–100 µg/L, there is evidence of adverse effects[10].

Arsenic poisoning may manifest as diseases ranging from neurological deficits to lung cancers. It has also been implicated in a wide range of dermatological problems, from mild keratosis to pigmentation changes [10].

Though some work has been done on the epidemiological levels of arsenic, the impact on human health is an area that still requires a lot of study. Key areas of exposure are residential areas near industrial estates with factories that use arsenic as a basic ingredient, e.g., pharmaceutical and paint factories in the industrial estate of Peshawar. Studies have shown that arsenic accumulates in keratin-rich tissue such as hair, skin, and nails due to a high affinity for sulfhydryl groups, and so these may be used as a biomarker in the detection of chronic poisoning [11,12].Thus, this study was conducted to find out the levels of arsenic in effluents and drinking water and the impact on the hair follicles of people working and living in this colony.

METHODOLOGY

The analysis was done in the Public Health laboratory Khyber Medical College and Institute of Basic Medical Sciences, Khyber Medical University, after approval from ASRB under: DIR/KMU-AS&RB001021/HS/KMC and Ethical Board under NO.596/ADR/KMC:DIR/KMU-EB/HE/ from June 2018 to April 2019. The sampling was done from the labor colony of industrial state Hayatabad, Peshawar. This colony consists of 200 houses near a pharmaceutical company. Sample size was calculated with the following assumptions;70% proportion of arsenic keratosis and hyperkeratosis among adults; with a 95% confidence Interval, & estimated sample size was n=243. Thus, to achieve this sample size, the houses were selected through a random number generator such that one factory worker and a non-factory worker were selected from each house along with a water sample, 81 in each category. In addition, water samples were also collected from the factory tube well, factory effluent and colony tube well. The study design was cross-sectional, as detailed in figure-I.

All the water samples were collected in clean, sterilized plastic bottles with an air-tight lid at three different intervals. A water sample of 100 ml was taken and mixed with 5ml of nitric acid in a sterilized beaker.

The sample was then placed on the hot plate for 3-4 hours on a slow flame (100°C) and then mixed with 100 ml of deionized water and filtered with Whatman filter paper. Finally, the solution was ready to be evaluated in an atomic absorption spectrometer. Hair samples from both the factory workers and their households (non-factory members) were also collected after informed consent. The samples were stored in a ziplock plastic bag to avoid external contamination for histological analysis and detection arsenic through atomic absorption spectrometer. After weighing a 0.1gm of hair sample using an analytical balance (Model No. QT 200), each sample was washed with ethanol to decrease external contamination without arsenic being leaked. These were decomposed by 8ml of nitric acid (HNO3) and were covered by a lid and placed on a hot plate at 70 – 85°C for 25 minutes until the solution became clear. After cooling the solution at room temperature, 30% hydrogen peroxide was added and again heated at 42°C. The heat was increased to a further 80°C till the volume was reduced to 2.5ml. The remaining solution was filtered by Whatman paper and transferred to a clean sterilized sample bottle to be analyzed in atomic absorption spectrometer.

Hair was taken from the sterilized plastic bag and cut into 2.2 mm to be placed on a glass slide. Next, we put two drops of DPX solution (mounting fluid) over it and covered it with a coverslip to make a wet mount. These were examined under light phase contrast microscope model (LH-M100CB-1, Nikon, Japan Eclipse 80i) at 400X by two experts and features such as colour, texture, pigment bodies, density and medullary index were noted in both factory workers and their household members. The medullary index was calculated as,

$$M.I = \frac{\text{Medullary diameter}}{\text{Shaft diameter}}$$

All data was analysed by using IBM SPSS version 20. Descriptive analysis was conducted for all the qualitative (hair morphometrics expressed as percentages) and quantitative variables (arsenic levels and medullary index of hair) presented by mean ± SD.

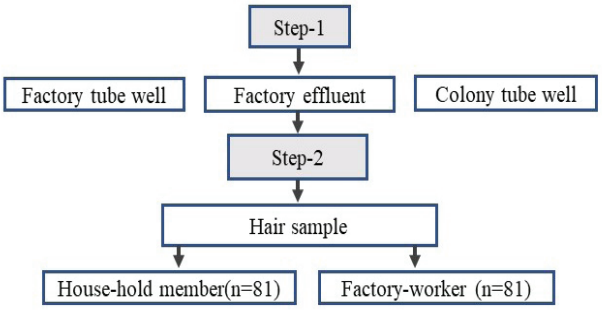

Figure-I: Study design and workflow.

RESULTS

This study was designed to observe arsenic levels in the drinking water and hair of factory workers and their household members and correlate them with hair histomorphology and skin lesions. The results obtained from this study were based on quantitative analysis of arsenic in water and hair including microscopic examination of hair samples from the scalp.

Demographic data collected showed a mean age of male factory workers ranging from 20 to 28 years, whereas non-factory working household members was 21 to 23 years.

The only co-morbidity identified was type-II diabetes mellitus in 6.2% of factory workers. History of hair loss in non-factory working household members was significantly more as compared to factory workers p<0.001(79 vs 19.8%).

As shown in (Table-I) levels of arsenic were consistently higher than the permissible level established by WHO.

The highest levels were in factory effluent with a 24.421ppb more than factory tube-well. When compared to the nearby colony tube-well, the levels decreased by 29.12ppb.

The arsenic levels of 81 household water samples decreased at a ratio of 1.75:1 (colony tube-well: household water samples). The household levels were in the range of 11.27 to 38.42ppb, all exceeding WHO criteria.

Table-I: Arsenic levels in water samples.

S. No.	Lab. Number	Arsenic Level (ppb)
1	Factory Tube Well	44.28
2	Factory Effluent	68.70
3	Colony Tube well	39.58
4	Household water	22.58 ±6.85

Table-II: Arsenic levels in a population under study.

Arsenic levels in hair samples (ppb)	Minimum	Maximum	Mean	Std. Deviation	p-value
House-hold Hair(n=81)	13.00	137.00	97.5	17.4	p≤0.001
Factory Worker (n=81)	83.00	183.00	131.8	24.6	

Hair samples were collected from each household as follows: a factory worker and a household adult member that was not a factory worker. The details are shown in (Table-II) for both groups. The difference was statistically significant (p≤0.001, independent sample t-test) between the two groups, the factory workers had relatively higher levels of arsenic, ~34ppb, in their scalp hair. The minimum ranges were also higher for the same group, ~70ppb.

Both qualitative and quantitative parameters of hair were determined histologically. Qualitative variables included hair colour, the texture of cortex and medulla, details of pigment bodies, cuticle layer (table-III, and figure:II-III). The commonest hair type in factory workers was black and in non-factory members was brown. We observed that the cortex of factory workers hair was coarser than that of household members (p≤0.05).

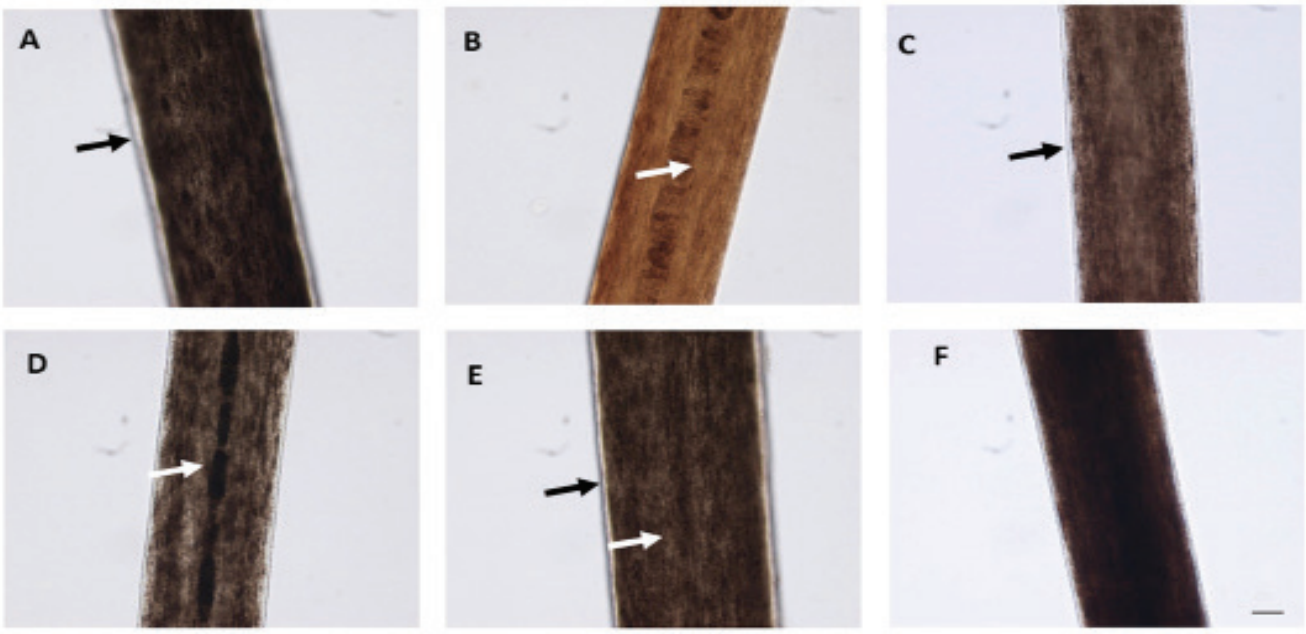

Figure-II: Representative images of qualitative parameters in the cortex, medulla and cuticle of hair in Factory workers (FW). Scale 20 µ. Black arrow Cuticle, White arrow Medulla.

A. Black smooth cuticle.
B. Cortical texture medium continuous medulla.
C. Abundant patchy pigment bodies absent cuticle.
D. Continuous medulla inner diffuse cuticle.
E. Medium pigment bodies, distinct cuticle.
F. Abundant heavy pigment bodies.

The quantity of pigment bodies was also more abundant, and denser and their degree of aggregation was patchier in factory workers than in household members.

Medulla was absent in approximately 34% of participants in both groups. More continuous medulla was observed in household member hair rather than factory worker hair (p≤0.05, chi-square). Medulla was more amorphous in factory workers as compared to household members (p≤0.05, chi-square).The outer margin of the hair cuticle was smooth in majority of household members and factory workers, but scales were observed only in household members (18.5%. p≤0.05, chi-square). The inner cuticle margin was more diffuse in the household members as compared to factory workers (p≤0.05, chi-square).

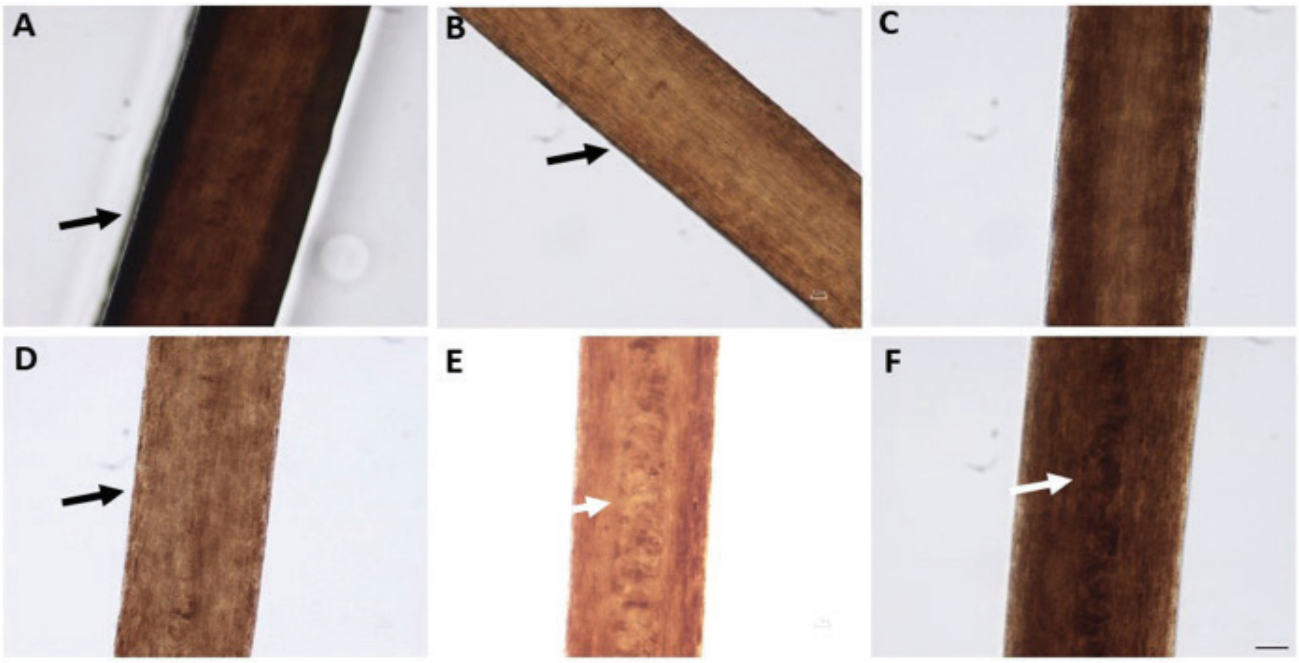

Figure-III: Representative images of qualitative parameters in the cortex, medulla and cuticle of hair in non-Factory workers (HH). Scale 20 micron. Black arrow Cuticle, White arrow Medulla.

A. Brown colour diffuse inner cuticle.
B. Brown medium cortical texture smooth cuticle.
C. Few patchy pigments bodies.
D. Absent medulla scaly cuticle.
E. Blonde medium pigment bodies.
F. Amorphous medulla.

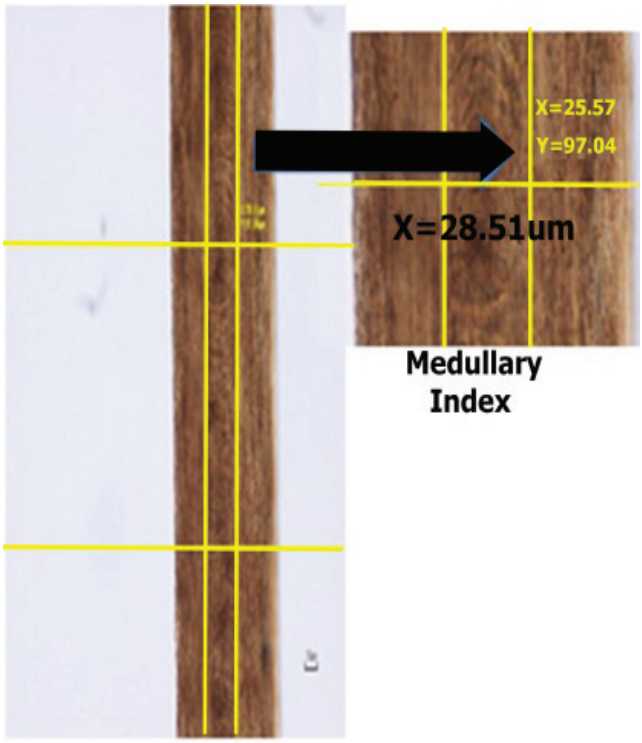

Figure-IV: Hair shaft with a calculation of the medullary index.

The quantitative variables were the shaft diameter and medullary index. A mean shaft diameter of 131.79±17.6 µm was observed in factory workers and 91.8±24.6 µm ($p \geq 0.05$, independent sample t-test) in non-factory workers.

The difference in the medullary index was 0.02µm and p-value= 0.005 in the two groups, which is significant as shown in (Table-IV and Figure-IV).

DISCUSSION

The toxicity of arsenic mainly depends upon its compounds and their valence; the inorganic trivalent form of arsenic being more toxic than organic arsenic. Its toxicity can cause various diseases like hypertension, cerebrovascular and respiratory diseases, and diabetes. Exposure to arsenic for a longer period can cause skin lesions which, if not treated in time, could lead to malignancies[10,13,14].

A rapid increase in urbanization and industrialization worldwide has led to people being more exposed to arsenic related morbidities. Not only developing countries like Bangladesh, India, Iran, Chile but also developed countries like the USA are facing threats to public safety due to high arsenic levels in water, most likely reason being the growth of their industries[15–17]. Pakistan, similarly, is facing the same threat due to the expansion of industries into more populated areas throughout the country. A recent study carried out by Podgorski JE et al, suggested that in the year 2014, about two hundred million people worldwide were exposed to high levels of arsenic i.e.,> 10µg/L of water, most effected countries were Bangladesh, India, Vietnam, and Nepal[8].

They measured arsenic levels in groundwater from 1200 wells in Pakistan and found that arsenic level was above 200µg/L along the Indus River valley at 3 to 70 meters.

Table-III: Comparison of qualitative parameters of hair in factory and non-factory workers.

Hair	Characteristics of Hair Samples	Morphology	Non-Factory n=81(%)	Factory Workers n=81(%)	p-value
Cortex	Colour	Black	15(18.5)	(38)46.9*	<0.001
		Brown	52(64.2)	(32)39.5	
		Blonde	14(17.3)	(11)13.6	
	Cortical Texture	Coarse	(10)12.3	(27)33.3	0.005
		Medium	(50.6)41	(35)43.2	
		Fine	37 (30)	(19)23.5	
	Quantity of Pigment bodies	Few	(43)53.1	(34)42	0.15
		Abundant	(38)46.9	(47)58	
	Aggregation of pigment bodies	Uniform	(10)12.3	(13)16	0.45
		Patchy	(70)86.4	(65)80.2	
		Streaks	(1)1.2	(3)3.7	
Medulla	Density of pigment bodies	Heavy	(5)6.2	(19)23.5	0.008
		Medium	(46)56.8	(37)45.7	
		Light	(30)37	(25)30.9	
	Medulla texture	Broken	(2)2.5	(9)11.1	0.02
		Fragmented	(9)11.1	(16)19.8	
		Continuous	(43)53.1	(28)34.6	
	Density of medulla	Absent	(27)33.3	(28)34.6	0.007
		Translucent (54)	(41)75.9	(27)50.9	
		Amorphous (53)	(13)24.1	(26)49.1	
Cuticle	Outer margin	Flat	(8)9.9	(13)16	0.018
		Smooth	(56)69.1	(64)79	
		Cracked	(2)2.5	0	
		Scales	(15)18.5	(4)4.9	
	Inner margin	Distinct	(9)11.1	(27)33.3	0.002
		Diffuse	(72)88.9	(54)66.7	

*All figures in bold indicate the maximum percentage of the characteristic, for example 46.9 (47%) of all Factory workers had black hair.

Table-IV: Medullary index of hairs.

Participant status	Minimum	Maximum	Mean±SD	p-value
Non-factory workers	0.14	0.31	0.21±0.045	0.005
Factory Worker	0.10	0.28	0.19 ±0.042	

In our study, we took four types of drinking water samples from the industrial estate which included samples from the factory tube well, factory effluent, labor colony tube well and 81 household tap water samples.

In all the samples, the arsenic level in drinking water was higher than 10ppb. This shows that although the levels are within the permissible range established by the Pakistan Council of Research in Water Resources for our country i.e. 50ppb; it is still higher than the level prescribed by the W.H.O (i.e. 10ppb).The effluent arsenic level in our study was even higher than 50ppb i.e. 68ppb, which means it is receiving arsenic contaminated dump from various factories which can come in contact with the underground water sources and could affect the people living in nearby residential areas or working in the factory located in the industrial estate area of Peshawar. Another similar study was conducted by M Ishaq et al, in which he analyzed mercury and arsenic in industrial effluents of Peshawar, in which they observed arsenic level was in the range between 10.8 to 66.4µg/L in factory effluents and arsenic level in drinking water in one of the pharmaceutical factory was also high i.e. 58.921µg/L, that is closely related to our findings as well[1] as was a study in industrial regions of District Sheikhupura in 2014[18].

A similar study was conducted by Niazi et al, also mentioned that 20% of population is exposed to a level up to 10µg/L and 3% had a level up to 50µg/L in Punjab[19]. The possible reasons for high arsenic in Punjab and Sindh provinces are probably the depth of tube wells (shallow tube wells) and the extensive industry network.

The second parameter in our study was the determination of arsenic level in hair which came out to be within the permissible range established by W.H.O i.e., < 1.00µg/g (1000ppb). Hair is an important biological parameter for determining chronic exposure to arsenic provided external contamination is excluded. As there is more keratin in hair and nails than in other parts of the body and the trivalent

inorganic arsenic has a greater affinity for sulpha-hydroxyl group in keratin hence higher deposits are found here.

Thus, hair might be considered an excretory pathway as once incorporated in the hair, arsenic is not biologically available. The organic form of arsenic known as Arseno-betain found in seafood can easily be excreted from the body and is not deposited in hair. This shows that arsenic in hair reflects exposure to inorganic arsenic only, and inorganic arsenic present in drinking water can be analyzed in hair and may be used as an indicator of exposure both in acute and chronic toxicity of arsenic[9,18]. Hindmarsh et al also reported strong correlation of arsenic in drinking water with that of arsenic in hair and other clinical manifestations[20]. Experimental studies in which radiolabeled DMA was administered to mice and rats showed very low incorporation of DMA in skin and hair compared with that of inorganic arsenic[21]. Thus, for deposits in hair, inorganic arsenic is the main component rather than any of its metabolites. Although ingestion of arsenic is a very important biological marker of chronic arsenic exposure but exogenous arsenic exposure either through bathing in arsenic contaminated water, shampoos, air pollutants, and soaps cannot be ruled out.

Moreover, attempts to remove externally deposited arsenic by washing may result in the removal of arsenic contained in the hair[22]. Therefore, although the determination of arsenic concentrations in hair might be useful for the detection of arsenic exposure, its use as an indicator of the degree of exposure to arsenic on an individual basis is limited.

In people with no known exposure to arsenic, the concentration of arsenic in hair is generally 0.02-0.2 mg/kg (it should be less than 1.00µg/g)[23].

Our third parameter was the determination of histological changes in hair with levels of arsenic in water. Hair morphology came out to be consistent with normal parameters of hair. The different layers of hair shaft were correlated with the hair features of other races so that a normal level could be designed. As per this research, limited data is available on arsenic's effects on hair histomorphology. An attempt is made to define the basic hair shaft parameters for further researchers to correlate these findings in studies carried out in the same area. Hair shaft was preferred over the hair root as mostly DNA studies are done on the root; secondly hair shaft can incorporate more arsenic than in hair root[9].

The cortex of hair shaft which consists of pigment bodies their density, degree of aggregation, texture all varies in different races e.g., in Asians, the cortex is round or oval, diameter of the shaft is somewhere between 20-175µm i.e., 0.06-0.17, the texture is usually coarser, and pigment bodies which impart colour to the hair can be densely packed or patchy depending upon the colour of hair. Cortex of Caucasians has moderate diameters, and pigment granules for thin and blonde hair are less in number and uniformly distributed. In African races, the findings are closer to our part of the world except that the shaft diameter is moderately increased, and pigment bodies are more densely packed and arranged in clumps[24]. In a study conducted in Uttar Pradesh, India, different types of medulla were noted, and all three types could be seen with the most fragmented patterns as opposed to our study of continuous in both groups[25].

Limitations:

A possible constraint was that only one labor colony and its associated pharmaceutical companies were evaluated for arsenic levels in water and hair. As the labor colonies have workers from other factories residing there, by bringing in all these factories located in the industrial estate Hayatabad Peshawar, more effective results regarding levels of arsenic can be ascertained.

CONCLUSION

The levels of arsenic in factory effluents in Hayatabad industrial estate are more than the prescribed safe level criteria determined by W.H.O, which is likely to cause a new health burden on the community in the coming years.

The levels of arsenic in the hair of factory workers and their non-factory household members came within the normal range of arsenic in hair i.e., <1.00µg/g, although the level in factory workers was significantly higher than the non-factory members, these levels need to be analyzed over a longer period of time to find out the chronic exposure of arsenic in these individuals.

The histomorphology of hair samples in factory and non-factory workers had normal Asian hair characteristics.

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Author's Contribution:

Mahvish Javed: Collected data, analysis, and drafting.
Zilli Huma: Study design, concept, interpretation and analysis.
Zeeshan Kibria: Study design, data analysis, and interpretation of data.
Muhammad Adeel Alam Shah: Data analysis, and drafting of the manuscript.

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