Identification of indicators of arsenic induced nephrotoxicity in humans
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Citation

Abstract
Arsenic contamination in drinking water is considered as one of the worst environmental health hazards leading to toxic effects on urinary bladder and other internal organs. Lack of comprehensive reports on the renal effect bio-indicators is the basis of our objective to assess/identify simple and cost-effective urinary effect parameters in relation to arsenic exposure. Increased incidence of urinary blood was seen with increasing concentration of arsenic in drinking water. Increased urinary protein, ketone and bilirubin appeared also in higher number of cases with rising concentration of arsenic. The subjects are exposed to arsenic which is nephrotoxic in the light of excretion of metabolites in urine. This was corroborated by exposure-induced rises in pigmentation and keratosis as clinical manifestation of nephrotoxicity.

INTRODUCTION
Arsenic toxicity due to drinking arsenic contaminated water has been one of the worst environmental health hazards (1). Water supply of some of the areas of nine districts of West Bengal are showing more than 50µg/L arsenic in ground water, which is more than World Health Organization’s maximum permissible limit of 50µg/L (2). The human health effects of arsenic toxicity ultimately lead to the development of arsenicosis. The major feature of arsenicosis is dermatological manifestations with diffused or spotted melanosis, leukomelanosis and keratosis. Epidemiologic evidence indicates that it is also carcinogenic to the urinary bladder and other internal organs (3). Urinary biomarkers for chronic arsenic exposure would be valuable as an early warning indicator for timely intervention (4). Studies also indicated that biological indicators, i.e., renal effect parameters [effect biomarkers], viz., Albumin in urine, is altered upon exposure of arsenic (5). Elevation of urinary porphyrins, viz., uro-porphyrins and copro-porphyrin III as warning biomarkers of chronic exposure among population in China has also been reported (6). Animal studies indicated that the severity of the renal lesion depended on the amount of nephrotoxin used (7). Nephrotoxin induced alteration of renal enzyme activity and urinary secretion is intimately related. Information on the renal toxicity of inorganic arsenic is available primarily from studies in animals using p-phenyl arsenic acid (8). The effect of heavy metals on kidney has already been established (9,10). An absence of in depth study of identification and characterization of damage in human induced by arsenicals is a shortcoming for development of specific biomarkers against this silent killer. The toxic insult on the renal system after arsenic exposure, if any, may open a new chapter in the field of arsenic toxicity. This area is still unexplored. The purpose of the present study is to assess/identify simple and cost-effective urinary effect parameters in relation to arsenic exposure.

MATERIALS AND METHODS
Sampling: A team of scientists visited the area of Katlamari Village, Murshidabad, West Bengal, India. The village is not only one of the most affected area in India but also in the world. Initially water samples were collected from the different drinking water sources and arsenic concentration of the water samples were estimated. List of the households of the gram panchayat were collected and the water sources used by the households was noted. A household may be using single water source or may be more than one. Based on the arsenic concentration of drinking water three categories have been made: a) subjects consuming water from the source with arsenic concentration ≤50µg/L, b) subjects consuming water from the source with arsenic concentration between >50µg/L & ≤150µg/L and c) subjects consuming water from the source with arsenic concentration >150µg/L. The houses were identified and number of subjects staying in one house with age of each subject was
noted. For the study, 100 exposed subjects were examined taking one subject from each family/household. A proportional allocation was made based on the population in each arsenic exposed category. Subjects were selected using random sampling techniques taking due of representation of age group (11). Thus 100 exposed subjects were covered for medical examination. Approval of the ethical committee at the Regional Occupational Health Centre (Eastern) was obtained for the study. Simultaneously written and informed consent was obtained from each of the participants.

Water Collection: Tube well water samples were collected in 100ml pre-washed polythene bottles (in 10% HNO3) and the samples were acidified with nitric acid (1ml/l). The water presently being consumed by the subjects were collected for each individual house for analysis purpose.

Estimation of arsenic in water: Estimation of arsenic in water was done by atomic absorption spectrophotometer (model-Avanta, GBC Scientific Equipment PVT. Ltd., Australia) attached with hydride generation system (GBC HG 3000, hydride generator) as previously described (12). Stock solutions of arsenic (1000mg/l) prepared from 99% AS2O3 (Sigma, USA) in deionised water and working standards were prepared daily from the stock solution. Before measurement all As present was reduced to As III by acidifying the samples with two molar hydrochloric acid (HCL) (2M HCl) and 0.2% potassium iodide (KI). Time period for complete reduction at room temperature was one hour. Arsenic concentration in the water samples was estimated against the working standards prepared daily from the stock solution at an absorbance at 193.7nm by using a AAS-HG technique.

Identification of urinary parameters: Fresh first excreting urine specimens were collected in a clean, dry containers in the morning. Specimens were mixed well immediately before testing. Urinary parameters were identified by using Bayer Reagent Strips for Urinalysis (M/s Bayer, Germany). The strips are firm plastic strips to which are affixed several separate reagents areas (13). Urinary blood tests were based on the peroxidase-like activity of haemoglobin, which catalyses the reaction of disisopropylbenzene dihydroperoxide and 3,3’, 5,5’-tetra methyl benzidine. Detection of urinary protein was between the reaction of 0.3% w/w bromophenol blue in a buffer system (of M/s Bayer) and the protein in urine. Urinary ketone was detected by the colour generation of reaction of nitroprusside with ketones. Bilirubin in urine was detected by the colour change of the coupling reaction of bilirubin with diazotized dichloroaniline in strong acidic medium. Principles of the processes are taken from manual of Multiple Reagent Strips for Urinalysis of M/s Bayer, Germany.

RESULTS
Detection of various urinary parameters were undertaken based on the concentration of arsenic in drinking water, which is categorized as follows: 1. ≤50µg/L, 2. >50µg/L and ≤150µg/L and 3. > 150µg/L. The percentage prevalence of cases in relation to excretion of various parameters are shown in Figure 1A, 1B, 1C, 1D.

Figure 1
Figure 1A: Effect of Arsenic on Urinary Blood

![Figure 1A: Effect of Arsenic on Urinary Blood](image)

Figure 2
Figure 1B: Effect of Arsenic on Urinary Protein

![Figure 1B: Effect of Arsenic on Urinary Protein](image)
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**Figure 3**
Figure 1C: Effect of Arsenic on Urinary Ketone

![Graph showing percentage of cases of urinary ketone](image)

**Figure 4**
Figure 1D: Effect of Arsenic on Urinary Bilirubin

![Graph showing percentage of cases of urinary bilirubin](image)

Studies indicated that, in males, blood appeared in urine in 10.6%, 23.6% and 17.7% cases with higher category of concentration of arsenic. The corresponding figures in female, were 22.7%, 36.9% and 35.4% respectively. Overall assessment of appearance of blood in urine was 17%, 30% and 27% of the tested population with corresponding concentration of arsenic as categorized earlier. Monitoring the appearance of cases of urinary protein showed that, in male, the figures were 73.75%, 76.5% and 76.5% corresponding to the arsenic concentration as described previously. In tested females, urinary protein was evident in 50%, 68.5% and 67.6% cases. Ketone bodies was detected in urine of 47.4%, 70.6% and 70.6 % examined males with corresponding to the arsenic concentration as described. The corresponding figures in females were 30.95%, 68.9% and 58.85% respectively. Appearance of urinary bilirubin was noted in 10.5%, 23% and 23.5% in tested males and in females, the figure was 45%, 26.5% and 23.5% with the corresponding concentration of arsenic in drinking water.

Dermatological examination for detection of exposure dependent pigmentation and keratosis of the subjects is shown in figure 2.

**Figure 5**
Figure 2: Effects of Arsenic on Clinical Symptoms

![Graph showing percentage of cases of pigmentation and keratosis](image)

Arsenic induced pigmentation was observed in 7.4%, 11.3% and 26.9% subjects of arsenic exposed to ≤50µg/L, >50µg/L and ≤150µg/L and >150µg/L respectively. The corresponding figures of keratosis were 8.6%, 9.7% and 21.2% respectively. Mainly the male subjects were victims of pigmentation and keratosis. A definite pattern of increase of skin pigmentation and keratosis was observed with the exposure of arsenic in male and total subjects. It has also been noted that the pigmentation in male and total subjects of water arsenic category >150 µg/L differed significantly when compared with that of water arsenic category ≤50µg/L. The corresponding figures of keratosis were 8.6%, 9.7% and 21.2% respectively. Mainly the male subjects were victims of pigmentation and keratosis. A definite pattern of increase of skin pigmentation and keratosis was observed with the exposure of arsenic in male and total subjects. It has also been noted that the pigmentation in male and total subjects of water arsenic category >150 µg/L differed significantly when compared with that of water arsenic category ≤50µg/L.

DISCUSSION

It has been observed that the biological indicators of renal effect parameters in both male and female, urinary blood, urinary protein, urinary ketone bodies and urinary bilirubin rise with increase in arsenic concentration in drinking water although the effect was not consistent.

Previous studies have shown that arsenic induces small
increases in urinary excretion of retinol binding protein (14). This was also found to be only dependable renal effect marker. Clinical studies indicated that cases of pigmentation and keratosis correspond with increasing arsenic concentrations in drinking water particularly in male. Similar type of changes in renal effect parameters induced by arsenic was observed in Chinese women (5). Arsenic is known to cause renal dysfunction by affecting tubular and glomerular impairment with simultaneous decrease in lysosomal latency as an early indicator of renal damage (8, 15). Studies on the nephrotoxicity of p-nitro phenyl arsenic acid using rat model also showed the elevated urinary protein over 8-day period following the injection of nephrotoxin (4). The discrepancy between the cases of renal effect parameters and that of clinical symptoms lies on the fact that elevation of renal effect parameters as warning biomarkers appears far before than appearance of clinical symptoms of arsenic exposure. In our study, the nephrotoxin induces renal injury as manifested by increased cases of urinary blood, protein, ketone and bilirubin with increase in arsenic concentration in drinking water. The study needs further investigation covering identification, characterization and quantification of urinary metabolites to establish a non invasive, and cost effective bio-indicator of arsenic induced renal injury in human.

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