

## Status of urinary porphyrins among population exposed to arsenic contaminated drinking water in arsenic endemic area of West Bengal, India

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**Abstract :** Arsenic contamination in ground water has been received much attention in recent times because of its carcinogenicity. Chronic exposure to arsenic through drinking water causes several multi-organ diseases including cancer. This study reports urinary porphyrins profile as a mark of disturbances in heme biosynthetic pathway due to chronic exposure to arsenic contaminated drinking water in endemic area of North 24-Parganas district of West Bengal. Arsenic in drinking water and urine were measured respectively by flow injection analysis system – Atomic Absorption Spectrometry (FIAS-AAS) and Transversely heated graphite atomizer (THGA-AAS) techniques. Urinary porphyrins were estimated simultaneously by reverse phase HPLC. Arsenic level in water was  $>50 \mu\text{g/L}$  in 57.7% tube wells, of which 13.3% were,  $>500 \mu\text{g/L}$ . Urinary arsenic ( $\mu\text{g/g}$  creatinine) was found to increase with increasing exposure. Study showed altered urinary porphyrins with changes in water arsenic (W-As) content as well as with duration of exposure. Higher porphyrin levels were observed in group, exposed to water arsenic,  $>250 \mu\text{g/L}$ , having exposure,  $\geq 15$  years, irrespective of gender. Significant differences between urinary porphyrins and duration of exposure of the subjects was noticed in the cases, uro III ( $p < 0.001$ ), penta I ( $p < 0.05$ ), copro III ( $p < 0.001$ ) and the ratio of copro III/uro III ( $p < 0.05$ ). An increasing trend of urinary porphyrins noticed with exposure to arsenic, though the difference between urinary porphyrins and exposure to water arsenic was insignificant. The study suggests that urinary porphyrins may serve as biomarker of chronic exposure to arsenic among population in the endemic area.

**Keywords :** Water arsenic, urinary arsenic, porphyrins, heme biosynthetic pathway, arsenic toxicity.

### Introduction

Arsenic toxicity is one of the major health problems in the world. West Bengal (India) and various other parts of the world, e.g. Bangladesh, Argentina, Taiwan, Mexico, Chile, USA, etc. are affected due to this problem and out of these West Bengal (India) and Bangladesh are mostly affected. Numerous studies have revealed association between prolonged ingestion of arsenic through drinking water and health outcome like, hyper-pigmentation, keratosis of skin, anemia, burning sensation of the eyes, solid edema of the legs, liver fibrosis, chronic lung disease, gangrene of the toes (Blackfoot disease), neuropathy and

cancers of skin, lung, liver, bladder, kidney, and prostate<sup>1-4</sup>.

Heme biosynthetic pathway is one of the most important pathways of human metabolism. Heme, a non-protein part of conjugated protein containing porphyrin combined with iron as the prosthetic group, in a wide variety of vital proteins (hemoproteins) e.g. hemoglobin, myoglobin, cytochromes, catalases, and peroxidases, is essential for biological oxidations<sup>5</sup>. Hemoproteins are responsible for oxidative reactions, electron transfer processes and the delivery of molecular oxygen to cells<sup>6</sup>. Since heme is involved in many important cellular processes, altered heme syn-

thesis results in a variety of harmful effects. The pathway begins in the mitochondria with the condensation of succinyl-CoA with glycine to produce ALA. ALA is then transported to the cytoplasm, where two molecules of ALA are condensed to form porphobilinogen (PBG). PBG are important to cellular processes due to their metal-binding capability. Four molecules of PBG are then converted into uroporphyrinogen followed by coproporphyrinogen. The decarboxylation and oxidation steps result in the conversion of coproporphyrinogen to form protoporphyrinogen in the mitochondria and finally to protoporphyrin. The final step involves the insertion of iron into protoporphyrin IX to form heme. Heme may be utilised in the formation of hemoproteins or degraded into bilirubin to excrete as bile pigment. Porphyrins are continuously excreted from the body either through the urine or as bile pigment and have no known biological function other than as precursors for heme<sup>7</sup>.

Heme, mainly synthesized in bone marrow (85%) where it is required for hemoglobin formation; the remaining 15% is synthesized in liver and other organs where it is required for hemoprotein synthesis<sup>8,9</sup>. It is reported that chronic exposure to environmental heavy metals (e.g. Hg, Pb, As) affect heme metabolism and as a result intermediates of heme biosynthetic pathway come into urine with heavy excretion<sup>10-12</sup>. Status of urinary porphyrins were studied by different scientists<sup>13-19</sup>. But which of the excreted intermediates are mostly caused due to exposure to arsenic were not very distinct. The present study focused on the intermediates of the heme biosynthetic pathway, affected mostly due to exposure to arsenic by showing change in porphyrin levels with change of exposure to drinking water arsenic. Besides, the study will show that the variation of urinary porphyrins with change in duration of exposure.

#### *Material and method :*

The study was conducted in a village, selected on the basis of preliminary pilot health survey, reported to be affected by ground water arsenic in North 24-Parganas district of West Bengal, India.

#### *Study subjects :*

The present study carried out during the period

2012-13. The study involved adult population, 62 (29 females and 33 males) from exposed village having reported cases of arsenicosis. The detailed history of water consumption was collected from the recruited subjects. Written signed consent from each participant (male and female) was taken before recruitment and as per the norms of Institutional Ethics Committee (IEC) of Regional Occupational Health Centre (Eastern), Kolkata under Indian Council of Medical Research (ICMR). The majority of the subjects recruited were occupationally rickshaw pullers, daily labourers and small business owners who were engaged in the surrounding area. Most of the subjects were illiterate and belong to poor socio-economical group.

#### *Reagents and standards :*

Uroporphyrin III dihydrochloride (uro III), heptacarboxyporphyrin I heptamethyl ester (hepta I), hexacarboxyporphyrin I hexamethyl ester (hexa I), pentacarboxyporphyrin I pentamethyl ester (penta I) and coproporphyrin III tetramethyl ester (copro III), mesoporphyrin IX dimethyl ester were purchased from Porphyrin Products (Logan, Utah, USA) for the estimation of porphyrins. HPLC grade glacial acetic acid was procured from Spectrochem, India for preparation of mobile phase and HPLC grade acetonitrile, ammonium acetate and methanol were bought from Merck, India for preparation of solvents. Arsenic(III), arsenic(V) standard (Sigma-Aldrich), Suprapur grade sodium borohydride (Sigma-Aldrich), Suprapur grade hydrochloric (Merck, Germany) and Suprapur grade nitric acids (Merck, Germany) were procured for the estimation of arsenic by AAS. Creatinine standard was purchased from Merck, India. HPLC grade water was used throughout the analysis. NaOH was obtained from Qualigen, KI, ascorbic acid and acetone were purchased from Spectrochem. Triton-X (2%), were obtained from Merck, Germany. Picric acid was obtained from Spectrochem.

#### *Environmental monitoring :*

Collection of drinking water samples : Study participants were provided with acid-washed (nitric acid-water (1 + 1)) plastic bottles for collection of drinking water (approximately 130 ml) samples into which

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hydrochloric acid (1.0 ml/L) was added later on as preservative. Water samples from tube wells of 45 nos. were collected and preserved in deep fridge at  $-20^{\circ}\text{C}$  until analysed.

**Arsenic estimation in drinking water :** Water samples were analysed for arsenic by using the AAS-FIAS (Perkin-Elmer-AA-800) at the absorbance wavelength 193.7 nm<sup>20,21</sup>.

#### *Biological monitoring :*

*Estimation of arsenic in urine and urinary porphyrins :*

**Collection of urine samples :** One time Spot urine samples were collected in 30 mL pre-washed polyethylene containers, added with 1% HCl as preservative, were collected during morning hours (not the first void) from the subjects in pre-washed polythene bottles and were kept in icebox immediately after collection. The samples were stored at  $-20^{\circ}\text{C}$  in the laboratory till analysed (and analysed within a week).

**Estimation of arsenic in urine :** The urine samples were digested in microwave digestion system (Ethos D, Milestone, USA) using nitric acid. Urine arsenic (U-As) was estimated as arsenate using THGA technique, using AA-800 attached with Graphite Furnace, Perkin-Elmer<sup>22</sup>.

**Measurement of creatinine :** Urine creatinine was determined by the standard Jaffe method<sup>23</sup>.

#### *Estimation of urinary porphyrins :*

Urinary porphyrins were estimated by reverse phase HPLC, Model SCL-10 AVP system, Shimadzu, Japan, having binary gradient pump (LC-10 AT vp), Rheodyne manual injector and a RF Xenon fluorescence lamp as detector<sup>16</sup>.

#### *Selection of column :*

A reverse phase C<sub>18</sub>, HPLC column (Radialpak, Novapak), 8×100 mm, 4 μm with a Novapak guard column, Water Associates, USA was used for estimation of urinary porphyrins.

#### *Selection of mobile phase :*

Two mobile phases A and B, were used for the analysis of porphyrins. The Phase A, consisted of a mixture of anhydrous ammonium acetate, acetonitrile

in HPLC grade water. The pH of the solution was adjusted to 5.16 with glacial acetic acid. In Phase B, 10% acetonitrile was mixed in methanol. The gradient programme according to Wang *et al.*<sup>16</sup> was adopted for the separation of porphyrins.

#### *Preparation of standards :*

Individual stock solutions for each of the porphyrins, uro III, hepta I, hexa I, penta I and copro III of concentration, 1 μmol/L were prepared separately. Stock solution of 1 μmol/L for the Internal Standard (IS) was also prepared similarly. Working standards were prepared from the above stock solution. Concentrations of porphyrins in working standards were from 1–100 nmol/L. All the stock of standard and IS were stored at  $-20^{\circ}\text{C}$ .

#### *Calibration and linearity :*

An eight-point calibration curve was prepared for porphyrins. Satisfactory linearity was observed with correlation coefficients ranging between 0.991 to 0.998.

#### *Sample preparation :*

The sample preparations were done as per the published method<sup>16</sup>. The stored samples were taken out and were kept outside to attain room temperature. In the first step, a mixture comprising 750 μL of urine and 50 μL of concentrated HCl was taken in an eppendrop tube. It was then vortex-mixed and was allowed to stand for almost 1 h in dark at room temperature. Then it was centrifuged at 10,000 r/min for 10 min. An aliquot of 20 μL was injected into the HPLC system for analysis.

#### *Statistical analysis :*

SPSS statistical software (Version 17) was used for the data analysis of the study. Student t-test was applied for the statistical comparison of concentrations of urine arsenic (U-As) and concentrations of porphyrins of male and female and their total at different W-As concentrations, duration of exposure.

## **Results and discussion**

Table 1 presents the distribution of the study subjects in the selected area of North 24-Parganas, West Bengal. The subjects were divided in the different

**Table 1.** Distribution of the subjects in selected study area according to age, duration of exposure and water arsenic exposure categories of North 24-Parganas, West Bengal

Characteristics					
Category of age (years)	<30	30 – <40	40 – <50	50 – <60	>60
Male	9	8	5	4	7
Female	6	10	7	4	2
Total	15	18	12	8	9
Category of duration of exposure (years)	≤5	>5–10	>10–15	>15	
Male	12	5	11	5	
Female	5	6	3	15	
Total	17	11	14	20	
W-As exposure (µg/L)	≤10	>10–50	>50–250	>250	
Male	6	7	7	13	
Female	4	7	8	10	
Total	10	14	15	23	

**Table 2.** Arsenic levels in tube well water at different concentration ranges of the study area of North 24-Parganas, West Bengal

	Arsenic levels in different water arsenic ranges (µg/L)				
	≤10	>10–50	>50–250	>250–500	>500
Mean ± SD	7.2±2.4	28.9±10.2	138.5±63.9	359.7±75.5	820.1±357.7
Range	(2.4–9.8)	(14.01–45.6)	(65.4–242.8)	(276.6–489.6)	(512.6–1515)
(Total, n = 45)	8 (17.8%)	11 (24.4%)	10 (22.2%)	10 (22.2%)	6 (13.3%)

categories of ages, like <30, 30 – <40, 40 – <50, 50 – <60, >60 years, exposures, like ≤ 5, >5–10, >10–15, >15 years and also according to exposure to different W-As levels, like ≤ 10, > 10–50, >50–250 and >250 µg/L.

Table 2 shows arsenic levels in water of tube wells in different concentration ranges of the study area of North 24-Paraganas, West Bengal. The distribution of tube wells (percentage) according to water arsenic (W-As) ranges showed that 17.8% of the tube wells in the study area were having W-As level, ≤ 10 µg/L and 24.4% having W-As, >10–50 µg/L. It was also observed that 22.2%, 22.2% and 13.3% tube wells of the study area, had W-As levels in the range, >50–250 µg/L, >250–500 and >500 µg/L respectively. Out of the total, 57.7% tube wells of exposed area showed higher W-As levels than the recommended national standard.

Table 3 presents urinary uroporphyrins and heptacarboxyporphyrins (nmol/g creatinine) excreted according to variation of W-As among population in

arsenic endemic area of West Bengal. Here W-As levels were divided in the following ranges, ≤ 10, >10–50, >50–250 and >250 µg/L respectively. It was observed that with increase in arsenic concentration in drinking water, urinary uro III gradually increases in case of male, female as well as in total. urinary hepta I gradually increases in case of male and total but for female subjects it gradually decreases.

Table 4 shows urinary hexacarboxyporphyrins and pentacarboxyporphyrins (nmol/g creatinine) excreted according to variation of W-As levels among population in arsenic endemic area of West Bengal. It was found that urinary hexa I and penta I gradually decrease in case of male, female as well as in total.

Table 5 presents urinary coproporphyrins (nmol/g creatinine) and copro III/uro III according to variation of W-As levels among population in arsenic endemic area of West Bengal. It was observed that with increase in arsenic concentration in drinking water, urinary copro III increases and ratio of copro III/uro III gradually decreases in all the category.

Table 6 shows urinary uroporphyrins and heptacarboxyporphyrins (nmol/g creatinine) excreted according to variation of duration of exposure (years), among population in arsenic endemic area of West Bengal. It was noticed that urinary uro III and hepta I gradually increased in almost all cases of male, female as well as in total. In case of uro III, the difference was observed to be significant ( $p < 0.001$ ).

Table 7 presents urinary hexacarboxyporphyrins and pentacarboxyporphyrins (nmol/g creatinine) excreted according to variation of duration of exposure (years), in population among arsenic endemic area of West Bengal. No steady change was however ob-

served in case of hexa I and penta I. Although the difference observed for penta I was significant ( $p < 0.05$ ).

Table 8 shows urinary coproporphyrins (nmol/g creatinine) and ratio of copro III/uro III according to variation of duration of exposure (years), among population in arsenic endemic area of West Bengal. It was observed that with increase in duration of exposure, urinary copro III and copro III/uro III ratio increases gradually. Fig. 1 shows a typical chromatogram of porphyrins in urine of arsenic exposed population of North 24-Parganas district, West Bengal, India.

**Table 3.** Urinary uroporphyrins and heptacarboxyporphyrins (nmol/g creatinine) excreted according to W-As among population in arsenic endemic area of North 24-Parganas, West Bengal

Porphyrins		Exposure range of W-As ( $\mu\text{g/L}$ )			
uro III		$\leq 10$	$> 10-50$	$> 50-250$	$> 250$
M		$9.0 \pm 5.6$	$14.9 \pm 10.3$	$11.8 \pm 9.0$	$16.0 \pm 8.5$
		6	7	7	13
	F	$10.3 \pm 1.9$	$9.5 \pm 4.5$	$17.2 \pm 9.9$	$15.9 \pm 6.9$
		4	7	8	10
	T	$9.5 \pm 4.4$	$12.2 \pm 8.1$	$14.6 \pm 9.6$	$16.0 \pm 7.7$
		10	14	15	23
hepta I	M	$2.1 \pm 0.4$	$4.4 \pm 3.3$	$2.9 \pm 2.4$	$5.1 \pm 5.7$
		6	7	7	13
	F	$6.4 \pm 5.8$	$2.4 \pm 1.9$	$4.4 \pm 3.4$	$4.0 \pm 3.2$
		4	7	8	10
	T	$3.8 \pm 4.0$	$3.4 \pm 2.8$	$3.7 \pm 3.0$	$4.7 \pm 4.7$
		10	14	15	23

**Table 4.** Urinary hexacarboxyporphyrins and pentacarboxyporphyrins (nmol/g creatinine) excreted according to W-As among population in arsenic endemic area of North 24-Parganas, West Bengal

Porphyrins		Exposure ranges of W-As ( $\mu\text{g/L}$ )			
hexa I		$\leq 10$	$> 10-50$	$> 50-250$	$> 250$
M		$0.8 \pm 1.9$	$0.1 \pm 0.1$	$0.1 \pm 0.2$	$0.5 \pm 1.3$
		6	7	7	13
	F	$1.5 \pm 2.8$	$0.4 \pm 0.9$	$0.3 \pm 0.4$	$0.6 \pm 1.7$
		4	7	8	10
	T	$1.1 \pm 2.2$	$0.2 \pm 0.6$	$0.2 \pm 0.4$	$0.6 \pm 1.5$
		10	14	15	23
penta I	M	$1.6 \pm 2.4$	$1.3 \pm 1.1$	$1.6 \pm 2.3$	$0.8 \pm 1.3$
		6	7	7	13
	F	$1.5 \pm 1.4$	$4.1 \pm 6.2$	$1.0 \pm 1.3$	$0.5 \pm 0.9$
		4	7	8	10
	T	$1.5 \pm 1.9$	$2.7 \pm 4.5$	$1.2 \pm 1.8$	$0.6 \pm 1.1$
		10	14	15	23

**Table 5.** Urinary coproporphyrins (nmol/g creatinine) and ratio of copro III/uro III according to W-As among population in arsenic endemic area of North 24-Parganas, West Bengal

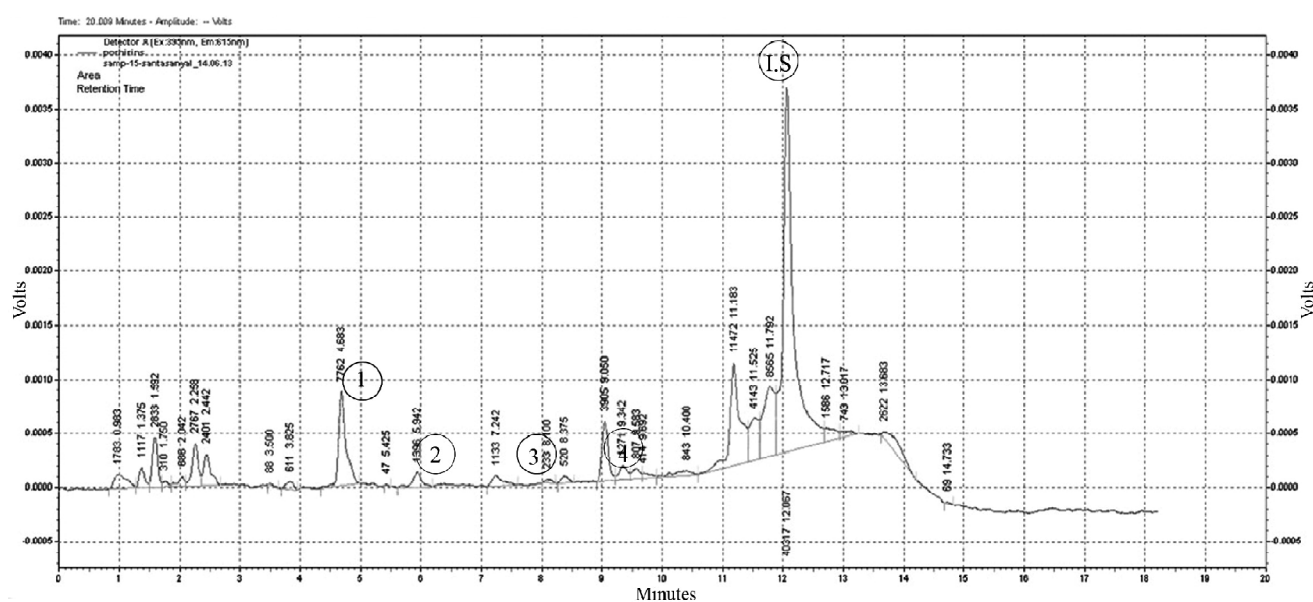
Porphyrins		Exposure ranges of W-As ( $\mu\text{g/L}$ )			
copro III	M	$\leq 10$	$> 10-50$	$> 50-250$	$> 250$
		$15.9 \pm 13.4$	$19.3 \pm 12.7$	$29.6 \pm 24.0$	$24.5 \pm 23.4$
	F	6	7	7	13
		$16.0 \pm 5.6$	$20.2 \pm 10.0$	$15.5 \pm 9.5$	$22.8 \pm 18.2$
	T	4	7	8	10
		$16.0 \pm 10.5$	$19.8 \pm 11.6$	$22.1 \pm 18.6$	$23.8 \pm 20.8$
	M	10	14	15	23
		$1.8 \pm 1.8$	$1.3 \pm 1.1$	$1.8 \pm 1.5$	$1.6 \pm 1.4$
copro III/uro III	F	6	7	7	13
		$1.5 \pm 0.4$	$2.6 \pm 1.9$	$1.2 \pm 1.3$	$1.3 \pm 0.8$
	T	4	7	8	10
		$1.7 \pm 1.4$	$1.9 \pm 1.6$	$1.5 \pm 1.4$	$1.5 \pm 1.1$
		10	14	15	23

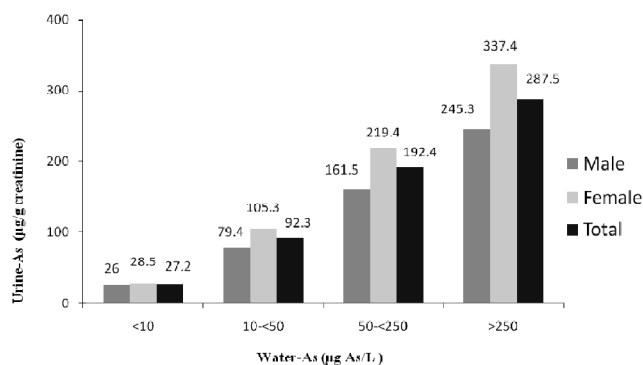
**Table 6.** Urinary uroporphyrins and heptacarboxyporphyrins (nmol/g creatinine) excreted according to duration of exposure (years), among population in arsenic endemic area of North 24-Parganas, West Bengal

Porphyrins		Duration of exposure (years)			
uro III	M	$\leq 5$	$> 5-10$	$> 10-15$	$> 15$
		$8.6 \pm 3.9$	$14.9 \pm 12.3$	$15.2 \pm 9.4^a$	$20.8 \pm 5.5^c$
	F	12	5	11	5
		$10.2 \pm 4.1$	$11.4 \pm 4.2$	$17.9 \pm 12.6$	$15.3 \pm 8.1$
	T	5	6	3	15
		$9.1 \pm 3.9$	$13.0 \pm 8.5$	$15.8 \pm 9.7^a$	$16.7 \pm 7.8^c$
	M	17	11	14	20
		$3.4 \pm 4.5$	$3.1 \pm 3.5$	$4.2 \pm 3.1$	$5.7 \pm 6.0$
hepta I	F	12	5	11	5
		$4.4 \pm 3.5$	$2.5 \pm 1.1$	$5.0 \pm 1.5$	$4.4 \pm 4.3$
	T	5	6	3	15
		$3.7 \pm 4.2$	$2.8 \pm 2.4$	$4.3 \pm 2.8$	$4.8 \pm 4.6$
		17	11	14	20

**Table 7.** Urinary hexacarboxyporphyrins and pentacarboxyporphyrins (nmol/g creatinine) excreted according to duration of exposure (years), among population in arsenic endemic area of North 24-Parganas, West Bengal

Porphyrins		Duration of exposure (years)			
hexa I	M	$\leq 5$	$> 5-10$	$> 10-15$	$> 15$
		$0.4 \pm 1.1$	$0 \pm 0$	$0.5 \pm 1.4$	$0.6 \pm 1.3$
	F	12	5	11	5
		$0.5 \pm 0.4$	$0.1 \pm 0.2$	$0 \pm 0$	$0.9 \pm 2.0$
	T	5	6	3	15
		$0.4 \pm 1.0$	$0.1 \pm 0.2$	$0.4 \pm 1.2$	$0.8 \pm 1.8$
	M	17	11	14	20
		$0.9 \pm 0.9$	$1.6 \pm 1.7$	$1.2 \pm 1.9$	$1.3 \pm 2.9$
penta I	F	12	5	11	5
		$3.7 \pm 5.8$	$1.1 \pm 1.1$	$4.6 \pm 7.0$	$0.6 \pm 0.9^a$
	T	5	6	3	15
		$1.7 \pm 3.3$	$1.3 \pm 1.3$	$1.9 \pm 3.5$	$0.8 \pm 1.6$
		17	11	14	20





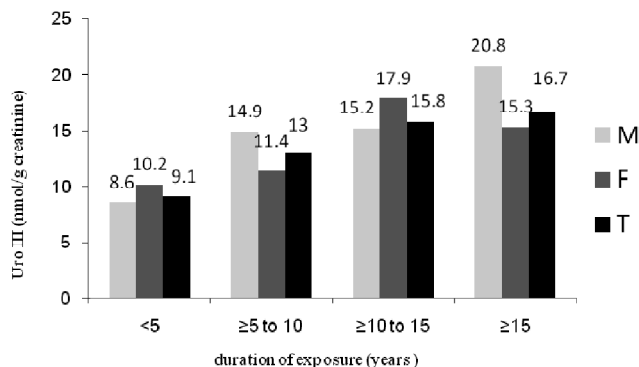
**Fig. 2.** Distribution of urine arsenic levels in µg/g creatinine according to water exposure (µg/L) in the endemic area of North 24-Parganas, West Bengal.

the exposed area which may be due to the fact that female subjects consume same water of the tube well throughout the day nearby home whereas male subjects usually go outside for their job and take water from different places away from home having different arsenic levels (may or may not contain arsenic).

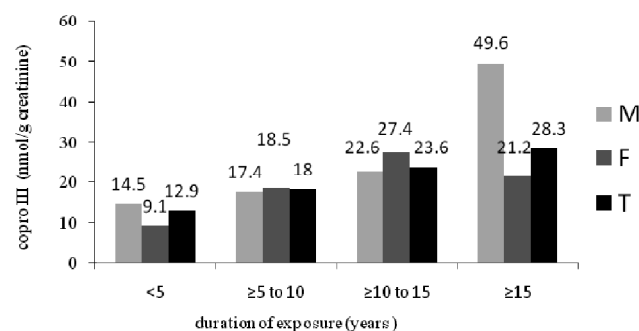
Fig. 3 presents urinary porphyrins (nmol/g creatinine) excreted according to duration of exposure (years), in population in arsenic endemic area of West Bengal. The urinary excretion of porphyrins mainly uro III, hepta I and copro III elevated with increased arsenic concentration in drinking water (Table 3 and Table 5), increased duration of exposure (Table 6 and Table 8) in case of male, female and also in total (combined male and female). These porphyrins exhibited highest values among the group exposed to water arsenic, > 250 µg/L, with exposure ≥15 years. Increasing tendency of the ratio, copro III/uro III was noticed with respect to variation of W-As but when compared with respect to duration of exposure, it was found to decrease gradually. No regular change in the urinary excretion pattern of hexa I, penta I was noticed in arsenic endemic area.

Garcia-Vargas<sup>15</sup>, studied the excretion of porphyrins in urine of human due to chronic exposure to arsenic through drinking water and reported significant reduction in copro III with increasing exposure of W-As in their study. The author also reported significant increase in uro III with increasing exposure to W-As. But in the present study, contrary to the

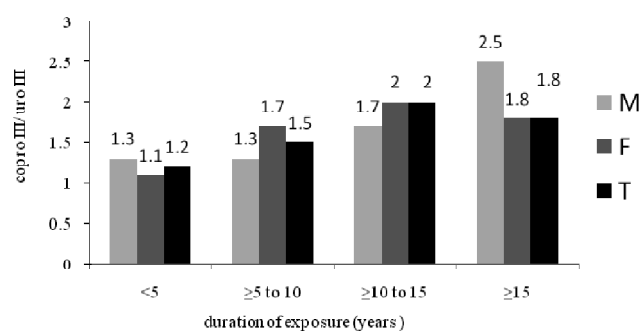
Variation of urinary uro III (nmol/g creatinine) excreted in population according to alteration of duration of exposure in arsenic endemic area of West Bengal.



Variation of urinary copro III (nmol/g creatinine) excreted in population according to alteration of duration of exposure in arsenic endemic area of West Bengal.



Variation of urinary copro III/uro III in population according to alteration of duration of exposure in arsenic endemic area of West Bengal.



**Fig. 3.** Urinary porphyrins (nmol/g creatinine) excreted according to duration of exposure (years), in population in arsenic endemic area of West Bengal.

later study, copro III was found to increase with exposure to W-As as well as increased duration of exposure. Besides, the present study also showed increase in uro III with increasing exposure to W-As as well as the duration of exposure.

Hernandez-Zavala<sup>12</sup>, studied the activities of enzyme of the heme biosynthetic pathway and its rela-



tion with change in urinary porphyrins of people exposed to arsenic chronically through drinking water in three selected areas of Mexico. The author reported increase in uro III, copro III as well as ratio of copro III/uro III, with exposure to arsenic. Our study however, differed from Hernandez-Zavala *et al.*, and reported increase in uro III, copro III as well as hepta I with increasing W-As exposure but decrease in the ratio, copro III/uro III with exposure to W-As but it was found that uro III, copro III, hepta I and the ratio, copro III/uro III increased with increasing duration of exposure.

Liu<sup>14</sup>, in their study on chronic arsenic poisoning due to arsenic contaminated ground water at Xing Xiang in China, reported increase in uro III and copro III with increasing exposure to W-As which supported the findings of our study. The results of uro III and copro III obtained by the author were found to be more or less similar to that of the present study. The results of porphyrins obtained in this study was found more or less similar to that reported by Ng<sup>13</sup>, Xie *et al.*<sup>27</sup>, in their studies of urinary porphyrins among populations exposed to chronic arsenic poisoning due to burning coal in China.

Elevated urinary levels of copro III and uro III, hepta I and decreasing tendency of copro III/uro III due to exposure to W-As observed in the present study revealed alteration in heme-metabolism among arsenic exposed individuals. Significant variations of porphyrins (mainly uro III, hepta I and copro III) was observed among the study population with increasing W-As exposure, duration of exposure in case of male, female as well as total. These variations show that although porphyrins, being a sensitive parameter and heme biosynthesis pathway is very active, the parameters of the pathway changes not only with exposure to W-As (Tables 3, 4 and 5) but also with duration of exposure (Tables 6, 7 and 8).

## Conclusion

The present study shows a progressive increase in urinary porphyrins mainly copro III, uro III and hepta I with increase in arsenic content in drinking water as

well as with duration of exposure to arsenic. The alteration in porphyrin status observed in the study focused the disturbances in the hemebiosynthesis pathway due to chronic arsenic toxicity among population exposed to arsenic in the selected area. Estimation of porphyrins in an arsenic endemic area are the newer aspects reported in the present study on the population of arsenic endemic area from West Bengal, India.

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