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Status of urinary porphyrins and inverse correlation of porphyrins with serum B vitamins in arsenic endemic area of West Bengal, India

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Abstract : Chronic arsenic toxicity through drinking water is still one of the major problems across the world. Bangladesh and India (particularly the state of West Bengal) are the worst affected countries with such problem. Millions of people all over the world are affected by arsenic related diseases. In our study, concentration of ground water arsenic, urine arsenic, hair arsenic and nail arsenic from adult individuals were measured from exposed areas of West Bengal, India respectively by flow injection atomic spectroscopy and transversely-heated graphite atomiser techniques. Porphyrins, uroporphyrin III, heptacarboxyporphyrin I, hexacarboxyporphyrin I, pentacarboxyporphyrin I, coproporphyrin III in urine were analysed separately by HPLC using fluorescence detector as well as level of vitamin B_6, vitamin B_9 and vitamin B_{12} were carried out in serum samples among the population in the selected area in North 24-Parganas, West Bengal, India. Concentration of uro III, hepta I, copro III and copro III/uro III were observed to increase irrespective of gender and also in total; whereas level of B vitamins were found to decrease with increase in exposure to arsenic contaminated drinking water from underground sources. The difference of vitamin B_6 ($p < 0.01$) between the population exposed to <50 and >50 μg/L arsenic in drinking water was found to be significant. Significant difference was also noticed in case of uro III ($p < 0.05$), copro III ($p < 0.01$) and copro III/uro III ($p < 0.05$) between two exposure categories. This study demonstrated increasing tendency in urinary porphyrins as well as inverse relationships of serum B vitamins with increased water arsenic concentration.

Keywords : Water arsenic, urine arsenic, hair arsenic, nail arsenic, porphyrin, vitamin.

Introduction

Human exposure to arsenic through contaminated groundwater is a serious health threat in many countries even now[1]. Consumption of the contaminated groundwater is likely to cause symptoms like weakness, conjunctival congestion, hyperpigmentation, keratosis, hepatomegaly, portal hypertension, respiratory system effects, polyneuropathy, solid oedema of limbs, and malignant neoplasms[2]. Arsenic is classified as a Group A and Category 1 human carcinogen by the US EPA and the International Association for Research on Cancer respectively[3,4]. B vitamins are essential for the function of vital biological systems such as cell production and energy production, so deficiency of B vitamins is very serious[5]. Vitamin B_6 serves as a cofactor in many enzyme reactions mainly in amino acid metabolism including biosynthesis of neurotransmitters[6]. Vitamin B_9 is involved in pyrimidine nucleotide synthesis, so is needed for normal cell division, especially during pregnancy and infancy, which are times of rapid growth. Folate also helps in erythropoiesis, the production of red blood cells. Vitamin B_{12} is involved in the cellular metabolism of carbohydrates, proteins and lipids. It is essential in the production of blood cells in bone marrow, and for nerve sheaths and proteins[7]. Vitamin B_{12} functions as a co-enzyme in intermediary metabolism for the methionine synthase reaction with methyl-

cobalamin, and the methylmalonyl CoA mutase reaction with adenosylcobalamin[8].

Heme biosynthetic pathway is one of the most important pathways of human metabolism. It is a complex eight step pathway involving a series of porphyrin and heme precursors. This synthesis proceeds from succinyl-CoA and glycine to uroporphyrinogen and in further series of steps to heme via pentacarboxy porphyrinogen and coproporphyrinogen. The heme biosynthetic pathway is tightly regulated by its end product, heme, through feedback inhibition; this ensures these porphryin precursors do not accumulate in excess under normal conditions. Porphyrins are chemical compounds made up of four pyrrole rings that are capable of binding metal ions and play essential roles in a variety of important biological reactions. Due to the ability of porphyrins to bind metal ions, they are capable of playing important roles in a variety of biological processes including O_2 transport (hemoglobin, myoglobin), photosynthesis (chlorophy ll), electron transfers (cytochromes) as well as drug metabolism (cytochromes P450). In humans, it is the porphyrin 'heme' that is utilised in myriad crucial metabolic pathways ranging from oxygen transport to gene regulation. Other than their role in heme synthesis, the porphyrin heme precursors have no known role in human metabolism and never accumulate under normal conditions as the heme pathway is tightly regulated. Each of the eight steps of heme biosynthesis is catalysed by a specific enzyme[9,10]. It has been evidenced experimentally that the heme synthesis pathway is highly active and susceptible to alterations induced by drugs and environmental chemicals (e.g. toxic heavy metals like As, Hg, Pb; pesticides etc.) in mammals leading to a change in porphyrin profile[11,12]. In the arsenic exposed area, intermediates of heme biosynthetic pathway come into urine in appreciable amount due to arsenic exposure than the unexposed. The specific porphyrin intermediates may serve as an early biomarker of chronic arsenic toxicity[13–16]. Only a few of these intermediates may be the potential biomarker of arsenic exposure.

It is evidenced that vitamin B_9 helps in the process of synthesis of glycine and vitamin B_6 helps in metabolism of glycine[17,18]. Folate may also act as a non essential activator of PBGD. It is also evidenced that folates activate uroporphyrinogen III synthatase[19]. Vitamin B_6 plays a major role in the hemebiosynthetic pathway as it is essential cofactor of the rate limiting ALAS enzyme of the heme synthesis[20,21].

According to study, it is known that biosynthesis of heme involves a variety of micronutrients like vitamin B_6, riboflavin, pantathenic acid, lipoic acid etc. The B vitamin deficiencies have been reported to correlate with increased porphyrin production[22]. Vitamin B_{12} helps in the conversion of methyl malonyl-CoA to succinyl-CoA which is required in the hemebiosynthetic pathway[23]. In this study, concentration of urinary porphyrins, uroporphyrin III (uro III), heptacarboxyporphyrin I (hepta I), hexacarboxyporphyrin I (hexa I), pentacarboxyporphyrin I (penta I), coproporphyrin III (copro III) and serum vitamin B_6, B_9 and B_{12} were analysed with the help of reversed phase HPLC method, concentration of arsenic in water, urine, hair, nail was measured by atomic absorption spectrophotometry. In our previous study, we have shown concentration of B vitamins gradually decreases with increasing concentration of arsenic in contaminated drinking water[24]. A few studies were also published on the urinary porphyrin profile due to chronic arsenic exposure through drinking water in arsenic endemic area as well as due to arsenic emitted from burning of contaminated coal[25–27].

In the present study, it will be focussed on the status of urinary porphyrin and serum B vitamins among the arsenic exposed population and their relationship. Besides, an inverse correlation between porphyrins and B vitamin with increased exposure to arsenic contaminated drinking water will be observed.

Materials and methods :

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 2010-11. The study involved adult population, 226

(119 females, having age range, 18–82 years and 107 males, having age range, 19–80 years) 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 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 economically poor families.

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. Standards of vitamins B_1, B_6 and B_{12} were procured from Merck, India. Standard compound of vitamin B_9 and internal standard (IS) theobromine were procured from Sigma-Aldrich, Switzerland. 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 was purchased from Spectrochem. Triton-X (2%,) was 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 hydrochloric acid (10 mL/L) was added later on as preservative. Water samples from tube wells of 148 Nos. were collected and preserved in deep fridge at –20 °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[28,29].

Biological monitoring :

Estimation of concentration of arsenic in urine, hair, nail and concentration of urinary porphyrins were carried out in the study.

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 of both exposed and control area in pre-washed polythene bottles and were kept in icebox immediately after collection. Then the samples were stored at –20 °C in the laboratory till 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[30].

Measurement of creatinine : Urine creatinine was determined by the standard Jaffe method[31].

Hair : Hair samples were taken from the subjects mainly from close to the scalp by using stainless steel scissor, cutting at a distance of *ca*. 1 cm from scalp and were kept individually in polythene packets at room temperature.

Estimation of arsenic in hair : The hair samples were digested in microwave digestion system (Ethos D, Milestone, USA) using nitric acid. Hair arsenic

(H-As) was estimated as arsenate using THGA technique, using AA-800 attached with Graphite Furnace, Perkin-Elmer[32].

Nail : Nail samples were collected from affected persons by nail cutter. After collection they were kept individually in polythene packets at room temperature.

Estimation of arsenic in nail : The nail samples were digested in microwave digestion system (Ethos D, Milestone, USA) using nitric acid. Nail arsenic (N-As) was estimated as arsenate using THGA technique, using AA-800 attached with Graphite Furnace, Perkin-Elmer[33].

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 fluoresence lamp as detector[26].

Selection of column :

A reverse phase C_{18}, 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[26] 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 °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[26]. 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.

Estimation of serum B vitamins :

Estimation of B vitamins (B_1, B_6, B_9, B_{12}) was undertaken in a sub-sample (n=33) only among the exposed area population by reverse phase HPLC of the same instrument as mentioned above using UV detector[34].

Selection of columns :

The Luna C18 (2) (150×4.6 mm, 5 mm) column, Phenomenex, Torrance, CA, USA, coupled with a guard pre-column, Phenomenex was used for the analysis of B vitamins. This was done under ambient oven temperature (25 °C).

Selection of mobile phase :

The mobile phase used for the estimation of B vitamins consisted of A : 0.05 *M* CH_3COONH_4/ CH_3OH (99/1) and B : H_2O/CH_3OH (50/50). The chromatographic separation of the vitamins was performed using the multi-step gradient programme for 30 min at a constant flow rate of 1 mL/min. The mobile phase and water used were degassed through 47 mm 0.2 μm, hydrophilic polypropylene membrane filters (Pall Corporation) before use. An interval of five minute was given as equilibration time between two injections. The chromatogram monitored at a wavelength of 242 nm.

Preparation of standards :

The standard stock solutions, 400 µM/L of vitamin B_1, 600 µM/L of vitamin B_6 and 100 µM/L of vitamin B_{12} were prepared in water and that of vitamin B_9, 250 µM/L, was prepared in an aqueous 1 (*M*) $NaHCO_3$ solution. A stock solution of theobromine as internal standard (IS), 500 µM/L, prepared in HPLC grade water was used. It was changed weekly. The working standards prepared for different vitamins were of concentrations, 0.5, 1.25, 2.5, 5, 10 and 50 µM/L. A working solution, 10 µM/L of IS, was prepared from the stock solution freshly on the day of analysis and added to the analyte during reconstitution after solid phase extraction (SPE)[34].

Calibration and linearity :

A six-point calibration curve was drawn with the standard mixtures of all the vitamins. All solutions were stored under refrigeration. The correlation coefficient of the calibration curve was found between 0.9963 and 0.9998.

Recovery :

The percentage recovery of each of the vitamin, B_1, B_6, B_9 and B_{12}, was observed at three different concentrations by comparing the peak area ratio of extracted vitamins to its respective standard value.

Preparation of sample :

The sample preparation was done with some modification in the technique by Chatzimichalakis[34]. The estimation of the B vitamin was done by using the standard addition method. Serum sample, 40 µL, mixed with 100 µL (1.25 µM/L) standard mixture of vitamins and 200 µL acetonitrile were taken in an Eppendorf tube and vortexed for 2 min, followed by centrifugation for 15 min at 4000 rpm. The Supelclean LC-18 cartridges (500 mg/3 mL) were first conditioned with 1 mL of methanol followed by 1 mL of deionised water. The supernatant after centrifugation was quantitatively transferred in the preconditioned SPE cartridge. The vitamins were eluted from the cartridge by 3 mL eluent, 0.05 (*M*) ammonium acetate in 85 : 15 v/v mixture of MeOH and water. The eluate from the cartridge was filtered through Swinney syringe with a 0.22 mm Millipore filter paper and washed thoroughly with 1 mL of eluent. The extracted solution was dried under vacuum evaporation. The dried residue was reconstituted by 100 µL aqueous solution (10 µM/L) of IS.

Exclusion criteria :

Subjects who were alcoholic, having problem with heart, pregnant women or using hormonal contraceptives were excluded from the study, since it may affect the concentration of homocysteine.

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 U-As, H-As, N-As, concentrations of porphyrins of male and female and their total at different W-As concentrations and concentration of B vitamins in serum of male, female and their total. A correlation study was observed among concentration of porphyrins and concentration of B vitamins.

Results and discussion

Fig. 1 presents the arsenic levels in water of tube wells in different concentration ranges of arsenic endemic area in North 24-Parganas district, West Bengal. The distribution of tube wells (percentage) according to water arsenic (W-As) ranges showed that 28.4% of the tube wells in exposed areas were having W-As level, ≤ 10 µg/L and 16.2% having W-As, >10–50 µg/L. It was also observed that 30.4%, 13.5%, 6.1% and 4.7% tube wells of the exposed

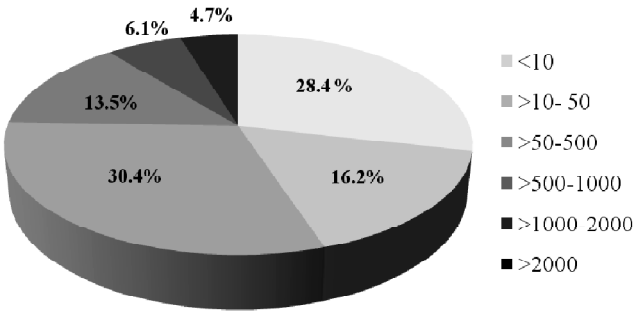

Fig. 1. Arsenic levels in water (µg/L) of tube wells in different concentration ranges of the study area of North 24-Parganas, West Bengal.

area, had W-As levels in the range, >50–500 μg/L, >500–1000 μg/L, >1000–2000 μg/L and >2000 μg/L respectively. Out of the total, 54.7% tube wells of exposed area showed higher W-As levels than the recommended national standard. The majority of the tube wells (30.4%) were having W-As levels, between, >50–500 μg/L with mean, 278.60±136.5 μg/L.

Figs. 2, 3 and 4 show concentration of arsenic in urine (μg/g creatinine), hair (mg/kg) and nail (mg/kg) samples obtained from study population in arsenic endemic area of West Bengal. Exposed samples were categorised with respect to W-As level who had exposed to, W-As ≤ 50, >50–500, >500–1000, >1000 μg/L respectively to observe the variation in U-As, H-As and N-As. It was found that with increase in arsenic concentration in water, U-As, H-As and N-As also increases significantly ($p < 0.0001$) in male, female and also in total (combined male and female).

Fig. 5 shows correlation analysis between urinary uro III and copro III with serum vitamin B_6 obtained

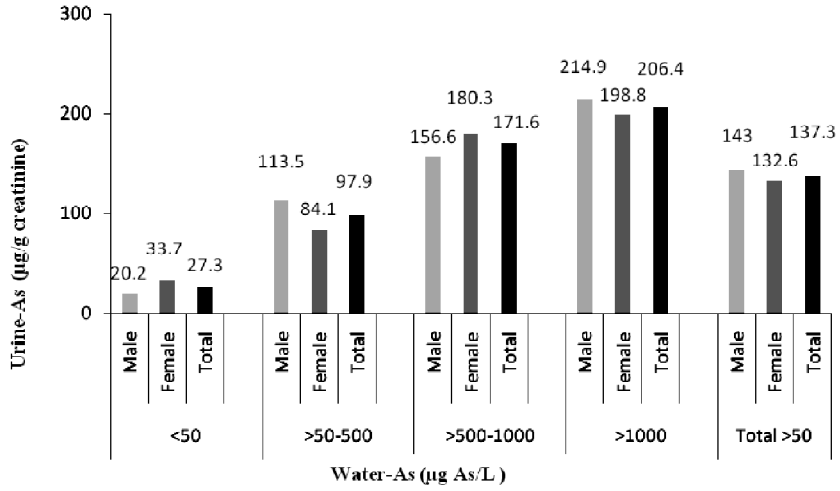

Fig. 2. Distribution of urine arsenic levels in μg/g creatinine according to arsenic exposure in drinking water in arsenic endemic area of West Bengal.

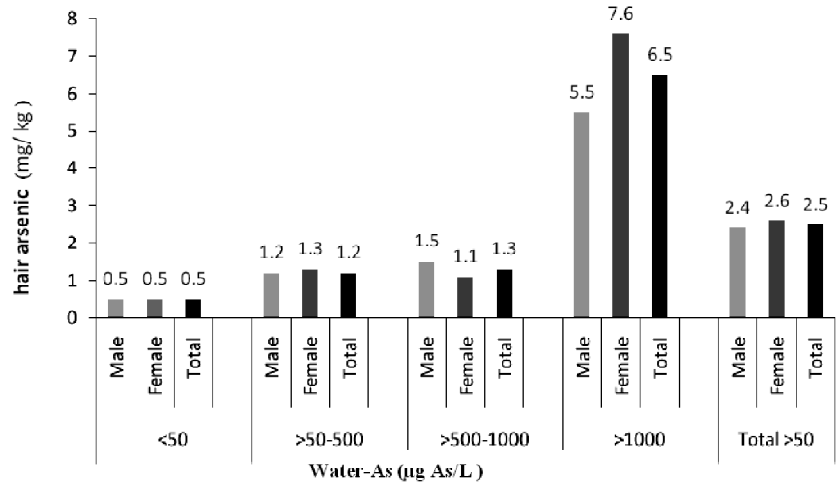

Fig. 3. Distribution of hair arsenic levels in mg/kg according to arsenic exposure in drinking water in arsenic endemic area of West Bengal.

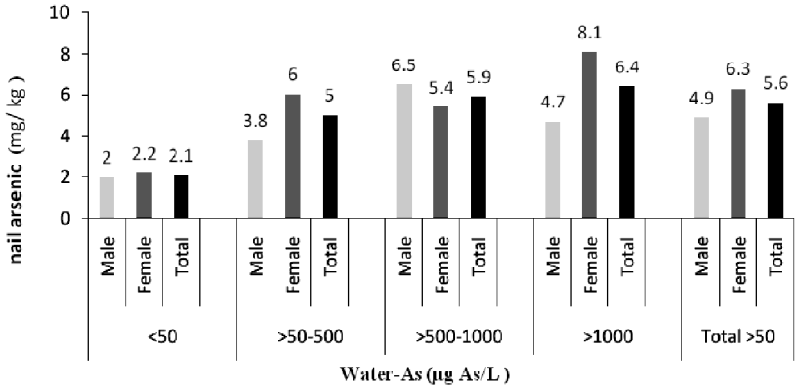

Fig. 4. Distribution of nail arsenic level in mg/kg according to arsenic exposure in drinking water in arsenic endemic area of West Bengal.

from study population in arsenic endemic area of West Bengal. It was observed that vitamin B_6 was inversely related with uro III and copro III among the population of the study area.

Fig. 6 shows correlation analysis between urinary uro III and copro III with serum vitamin B_6 obtained

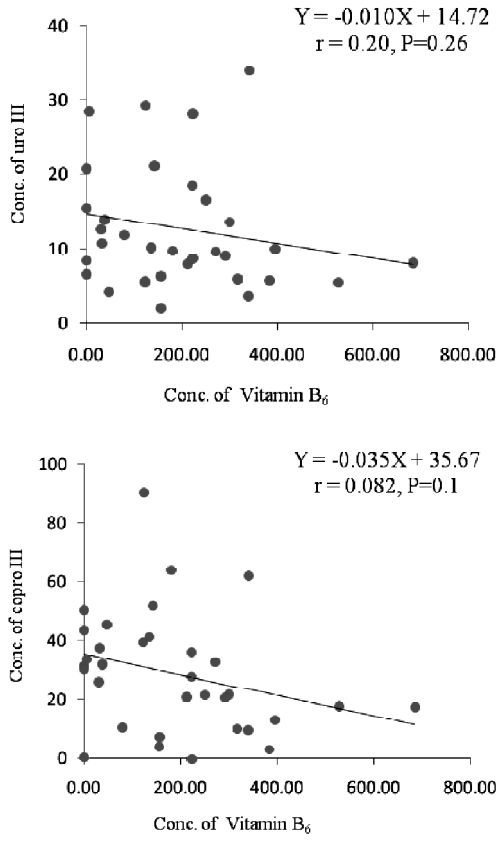

Fig. 5. Correlation analysis between vitamin B_6 (nM/L) and uroporphyrine III, coproporphyrine III (nmol/g creatinine) obtained from study population in arsenic endemic area of West Bengal.

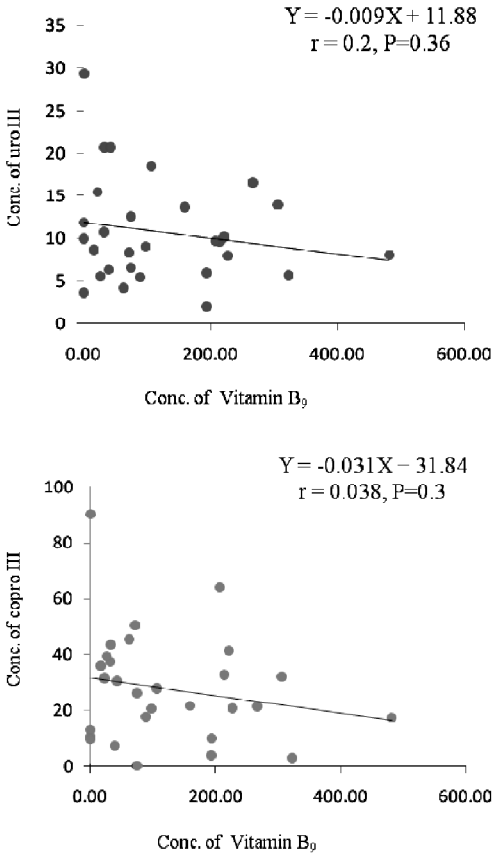

Fig. 6. Correlation analysis between vitamin B_9 (nM/L) and uroporphyrine III, coproporphyrine III (nmol/g creatinine) obtained from study population in arsenic endemic area of West Bengal.

from study population in arsenic endemic area of West Bengal. It was also observed that vitamin B_9 was inversely related with uro III and copro III among the population of the study area.

Fig. 7 shows correlation analysis between urinary copro III and copro III/uro III with serum vitamin B_{12} obtained from study population in arsenic endemic area of West Bengal. It was also observed that vitamin B_{12} was inversely related with copro III and copro III/uro III among the population of the study area.

Table 1 presents concentration of vitamin B_6, vitamin B_9, vitamin B_{12} (nM/L) and concentration of urinary porphyrins, uro III, hepta I, hexa I, penta I, copro III (nmol/g creatinine), copro III/uro III obtained from study population distributed according to variation of concentration of W-As of the study area

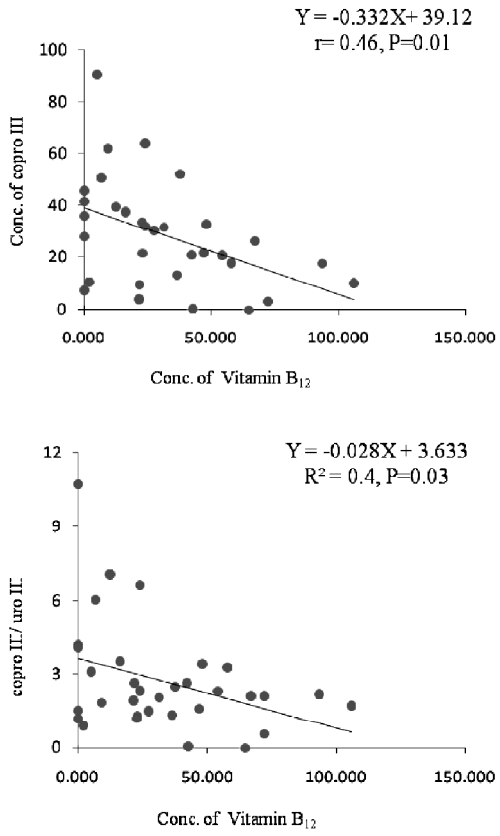

Fig. 7. Correlation analysis between vitamin B_{12} (nM/L) and coproporphyrine III (nmol/g creatinine) and copro III/ uro III obtained from study population in arsenic endemic area of West Bengal.

of North 24-Parganas, West Bengal. It was observed that with increase of W-As, concentration of vitamin B_6, vitamin B_9, vitamin B_{12} decreases. Although significant difference was observed in case of only vitamin B_6 ($p < 0.01$). In case of porphyrins, uro III, copro III and copro III/uro III increases with increasing W-As concentration. Significant difference was observed in case of uro III ($p < 0.05$), copro III ($p < 0.01$) and copro III/uro III ($p < 0.05$).

Table 2 presents concentration of arsenic in urine (μg/g creatinine), hair (mg/kg) and nail (mg/kg) of the selected subjects obtained from study population distributed according to variation of concentration of W-As of the study area of North 24-Parganas, West Bengal. It has been found that concentration of urine, hair and nail increases with increasing W-As concentration. Significant difference was observed in case of urine ($p < 0.05$), hair ($p < 0.0001$) and nail ($p < 0.001$).

In this study, U-As was used as a bioindicator of recent exposure, H-As and N-As as bioindicator of long term exposure for population of the arsenic endemic area of West Bengal. The study also observed increasing tendency of urinary porphyrins as well as decreasing tendency of B vitamins with increasing water arsenic exposure in population in arsenic exposed area in Barasat, North 24-Parganas, West Bengal. Further, the urinary porphyrin levels were compared with the B vitamin level among the sub-populations of the arsenic endemic area.

Concentration of arsenic in urine, hair and nail was found to increase with increased arsenic concentration in drinking water (Figs. 2, 3 and 4). Concentration of arsenic in urine, hair and nail was found more for female subjects compared to male in most cases which indicate that female subjects are more affected due to arsenic exposure due to staying long time in home while male subjects usually go outside for job purposes.

The urinary excretion of porphyrins mainly uro III and copro III and copro III/uro III elevated with increased arsenic concentration in drinking water (Table 1), in case of male, female and also in total (combined male and female). These three parameters

Table 1. Concentration of vitamin B_6, vitamin B_9, vitamin B_{12} (nM/L) and concentration of urinary porphyrins, uro III, hepta I, hexa I, penta I, copro III (nmol/g creatinine), copro III/uro III obtained from study population distributed according to variation of concentration of W-As of the study area of North 24-Paraganas, West Bengal

W-As level	n		Vitamine B_6	Vitamine B_9	Vitamine B_{12}	uro III	hepta I	hexa I	penta I	copro III	copro III/uro III
<50	8	M	325.2±159.9	208.1±143.9	49.5±40.1	10.5±5.1	0.5±0.8	0±0	0.1±0.2	16.4±8.5	1.5±0.6
	9	F	220.4±180.2	106.8±95.4	28.8±18.7	9.8±7.7	1.5±1.7	0±0	0±0	21.5±14.8	2.6±1.6
	17	T	269.7±174.1	154.5±127.7	38.5±31.5	10.1±6.4	1.0±1.4	0±0	0±0.1	19.1±12.2	2.1±1.3
>50	9	M	105.9±83.2^b	105.0±107.5	25.5±26.6	12.3±7.6	2.7±3.2	0.1±0.2	0.7±1.5	34.5±14.9^b	4.0±3.2^a
	7	F	97.5±128.2	139.4±133.5	29.2±22.6	19.3±10.0^a	2.1±3.3	1.3±3.1	0.3±0.7	46.2±29.0^a	2.5±2.0
	16	T	102.3±101.4^b	120.1±116.7	27.1±24.2	15.7±9.0^a	2.4±3.1	0.6±2.1	0.5±1.2	39.6±22.2^b	3.4±2.8

Table 2. Concentration of arsenic in urine (μg/g creatinine), hair (mg/kg) and nail (mg/kg) of the selected subjects obtained from study population distributed according to variation of concentration of W-As of the study area of North 24-Paraganas, West Bengal

W-As level	n		Urine-As	Hair-As	Nail-As
<50	8	M	17.2±26.8	0.2±0.2	1.5±1.6
	9	F	24.8±65.8	0.3±0.5	2.2±1.4
	17	T	21.3±49.9	0.3±0.4	1.9±1.5
>50	9	M	69.8±83.3	8.4±7.0^b	5.0±4.2^a
	7	F	325.0±300.8^a	8.0±8.7	10.0±4.5^c
	16	T	181.5±238.7^a	8.2±7.5^d	7.0±4.9^c

^{a}Significance test between non exposed and exposed population; $^a p < 0.05$, $^b p < 0.01$, $^c p < 0.001$, $^d p < 0.0001$.

M = Male, F = Female, n = Total no. of samples.

exhibited higher values among the group exposed to water arsenic, >50 μg/L. No regular change in the urinary excretion pattern of hepta I, hexa I, penta I and copro III/uro III was noticed in arsenic endemic area. Concentration of vitamin B_6, vitamin B_9, vitamin B_{12} in serum decreases with increased arsenic concentration in drinking water (Table 1). These three parameters exhibited lower values among the group exposed to water arsenic, >50 μg/L.

In 1994, Garcia-Vargas and coworkers[14], studied the excretion of porphyrin in urine of human due to chronic exposure to arsenic through drinking water. Hernandez-Zavala[15], studied the activities of enzyme of the heme biosynthetic pathway and its relation with change in urinary porphyrin levels of people exposed to arsenic chronically by drinking water in three selected areas of Mexico. Liu[13], also studied chronic arsenic poisoning due to arsenic contaminated ground water at Xing Xiang in China. Similar studies were carried out by Wang[27], Xie[25]. In 2016, Mukherjee and his co-researchers have estimated vitamin level among population in arsenic exposed area in Barasat, North 24-Parganas, West Bengal[24]. But no one has reported the level of porphyrin and B vitamins among population in arsenic exposed area. Elevated urinary levels of uro III, copro III and copro III/uro III was observed in the present study reveal that there is disturbances in heme-metabolism among arsenic exposed individuals. Significant variations of porphyrins (mainly uro III and copro III) was observed among the study population with increasing W-As exposure male, female as well as total. These variations show that although porphyrins being a sensitive parameter and hemebiosynthetic pathway is very active pathway, the parameters of the pathway changes with exposure to arsenic contaminated water (Table 1).

Conclusion

The study shows that concentration of urinary porphyrins increases with W-As exposure. It also shows level of B vitamins gradually decreases with increasing W-As exposure. A negative correlation of urinary porphyrin and serum B vitamin was observed in our study. Arsenic concentration in biological sample like urine, hair and nail among arsenic exposed individuals in arsenic endemic area were found to increase as W-As exposure increases. The study of porphyrin in West Bengal was made by our team before, but estimating porphyrin and B vitamin in a single study was never done before. This study may be extended in future with larger sample size.

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