

Identification of indicators of arsenic induced hepatic damage in human

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Abstract

Arsenic (As) exposure through contaminated drinking water is one of the environmental health hazards leading to toxicity as manifested by symptoms and signs. Little information is available on the hepatotoxic behaviour of arsenic (As) in exposed human. The aim of the present study is to assess bio indicators of hepatocellular injury, cholestatic injury, liver biosynthetic capacity, diabetogenic probability of arsenic in relation to clinical manifestation of exposed subjects and compare it with control. One hundred exposed individuals ($121.95 \pm 11.30 \mu\text{g/L As}$) and 50 control subjects ($15.17 \pm 2.07 \mu\text{g/L As}$) of comparable age were studied. Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALKP) increased significantly ($p < .001$) in tested exposed individuals with respect to control. Elevated level of serum globulin was observed in exposed subjects in comparison to control. Rising trend of blood glucose was exhibited in tested population although it was within normal range. Level of serum urea in females were elevated upon exposure but was found within normal limit. Palpability of liver was shown to increase in exposed population drinking arsenic contaminated water. Exposure-induced rise in pigmentation and keratosis was also noted. Clinically detected arsenicosis cases showed higher values of serum AST, ALT, ALKP, and Urea. Our study reveals that arsenic exposure generates stress on the liver indicated by elevation of bioindicators including hepatic enzymes.

INTRODUCTION

Arsenic (As) is a well documented human toxicant and contamination with this heavy metal is a global concern presenting a major issue in environmental health⁽¹⁾. Recent studies suggested that a vast area of 9 districts of West Bengal, India is severely affected by this toxicant of geological origin and the exposed population is about 42.7 million⁽²⁾. Besides dermatological manifestations like pigmentation and keratosis, its exposure is associated with various systemic manifestations⁽³⁾. Arsenic induced non-cirrhotic portal hypertension in two patients was first reported from Chandigarh⁽⁴⁾. It was also reported that non-cirrhotic portal lesion is the predominant lesion in chronic arsenic toxicity⁽⁵⁾. Exposure of arsenic through drinking of contaminated water may cause conjugated hyperbilirubinemia with simultaneous rise in serum alkaline phosphatase (ALKP)⁽⁶⁾. Elevation of ALKP, AST and ALT was noticed as an effect of arsenic exposure in human⁽⁷⁾. Our laboratory also observed arsenic induced stress which may cause hepatic injury. Little information is available on stress which mirrors such damage in hepatic tissues at risk of arsenic exposure. The studies of arsenic toxicity involving

human subjects were carried out only on exposed individuals although reports of arsenic induced hepatic toxicity are available on murine model using control. The aim of the present study is to assess bio indicators of hepatocellular injury, cholestatic injury, liver biosynthetic capacity, diabetogenic probability in relation to clinical manifestation of the arsenic exposed subjects and compare it with control.

MATERIALS AND METHODS

Sampling: A team of scientists visited the area and chosen five exposed and two control wards out of 22 wards of Ashokenagar- Kalyangarh municipal area of 24 Paraganas, West Bengal, India. This district was chosen for its proximity to Kolkata, the consequent convenience of transporting samples from the study site to the laboratory and also because it is reported to be severely affected by arsenic⁽⁸⁾. For the study, it has been decided to examine 150 exposed and 50 control subjects. A proportional allocation was made taking one family as unit depending on the population of ward. Basing on the proportional allocation, adult males and adult females, males below 25 years and females below 25 years were chosen using random sampling⁽⁹⁾ taking one family as one unit. Thus 150 exposed subjects

and 50 control subjects were covered for medical examination. Average duration of consuming arsenic contaminated water for tested population was 7-8 years. 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 used by these subjects was also collected.

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⁽¹⁰⁾. 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.

COLLECTION OF SERUM AND PLASMA SAMPLES

Each sample of blood was collected in clean sterile vial and divided into two tubes: one (1 ml) containing fluoride for plasma separation and the other (1 ml) was allowed to clot for serum separation.

BIOCHEMICAL EVALUATION

From serum samples of exposed subjects, the activities of aspartate transaminase (AST)⁽¹¹⁾, alanine transaminase (ALT)⁽¹²⁾, and alkaline phosphatase (ALKP)⁽¹³⁾ were determined. The concentration of urea was also assayed in serum⁽¹⁴⁾. The sugar content of the samples in plasma was determined according to the method described by Trinder et al⁽¹⁵⁾. All the assays of exposed subjects were carried out with Chemwell analyser (USA). Serum Total protein and albumin was estimated by the method of Striclad et. al⁽¹⁶⁾

and Doumas et. al⁽¹⁷⁾ respectively.

STATISTICAL ANALYSIS

Values were expressed as the mean \pm SD and the significance of the differences between mean values were determined by Student's t test.

RESULT

Mean arsenic concentration in drinking water was detected 15.17 \pm 2.07 μ g/L in control population (range 0.01-44.44) and 121.95 \pm 11.30 μ g/L (range 59.26-190.81) in exposed population. Average age group of tested males and females were as follows:

Exposed : male (>25 years) = 45.94 years; female (>25 years) = 42.47 years; Control male (>25 years) = 50.25 years; female (>25 years) = 47.94 years; Exposed male (\leq 25 years)= 19.00; female (\leq 25 years)= 17.89 years; Control male (\leq 25 years) = 17.50 years; female (\leq 25 years) = 14.20 years [unpublished data].

Table 1 represents the percentage of cases showing above normal range of biochemical parameters. It has been noticed that about 60% of both male (65%) and female (55%) exposed subjects of age group \leq 25 years showed higher values of AST. About 82% of both male (81%) and female (83%) exposed subjects of age group > 25 years showed higher values of AST. ALT showed similar pattern in tested population. Our study indicated that ALKP level increases with increase in age (29% at \leq 25 years and 32% at > 25 years) in case of tested male. The corresponding figure was 20% (at \leq 25 years) and 28% (at > 25 years) in case of female. It was observed that the enlargement of livers showed some similarity with it.

Figure 1

Table I: Percentage of cases of arsenic exposure induced deviation from normal range of biochemical parameters

	Male		Female	
	Age \leq 25 years (n=17)	Age >25 years (n=53)	Age \leq 25 years (n=20)	Age > 25 years (n=61)
Glucose	18	13	40	2
Serum AST	65	81	55	83
Serum ALT	47	58	25	65
Serum ALKP	29	32	20	28

Table 2A indicates the effect of arsenic exposure on biochemical parameters of male. AST and ALT, as indicator of hepatocellular injury, of exposed subjects of both age group increased significantly ($p < 0.001$) in comparison to control. ALKP, as biomarker of cholestatic injury, showed similar trend. Total Protein, as indicator of liver biosynthetic

capacity, of the tested males elevated significantly in comparison to control but was within normal range. Albumin of both the age group declined significantly ($p<0.001$) compared to control resulting in increase in quantity of globulin. Urea and blood glucose of exposed males (age group > 25 years) elevated significantly ($p<0.001$) with respect to control.

Figure 2

Table 2A: Effect of arsenic exposure on biochemical parameters in males

Biochemical parameters (unit)	Age Group					
	Control (n=6)	Exposed (n=17)	Change (P value)	Control (n=19)	Exposed (n=53)	Change (P value)
	Age ≤ 25 years	Age ≤ 25 years		Age >25 years	Age >25 years	
Glucose (mg/dL)	79.75 \pm 4.25	78.41 \pm 3.98	NS	77.80 \pm 1.30	84.36 \pm 2.36	0.001
Serum AST (U/L)	33.33 \pm 9.99	110.94 \pm 28.21	0.001	36.68 \pm 3.97	122.22 \pm 10.53	0.001
Serum ALT (U/L)	49.40 \pm 11.94	84.92 \pm 20.92	0.001	38.74 \pm 5.74	86.60 \pm 9.06	0.001
Serum ALKP (U/L)	99.50 \pm 29.04	198.64 \pm 25.47	0.001	72.10 \pm 28.30	148.49 \pm 3.36	0.001
Serum Total Protein (g/dL)	7.15 \pm 0.24	7.92 \pm 0.11	0.001	7.44 \pm 0.12	7.76 \pm 0.08	0.001
Serum Albumin (g/dL)	5.23 \pm 0.10	5.01 \pm 0.08	0.001	5.13 \pm 0.05	4.79 \pm 0.06	0.001
Serum Urea (g/dL)	19.31 \pm 1.53	20.20 \pm 1.45	NS	17.23 \pm 1.24	20.33 \pm 0.77	0.001

Values are mean \pm SD

Number in parenthesis indicating subjects

Table 2B indicates the effect of arsenic exposure on biochemical parameters of female. Significant elevation ($p<0.001$) of AST, ALT and ALKP was noted compared to control population. Increase in TP was observed in case of tested females of age group ≤ 25 years in comparison to control. Alb decreased significantly [age group ≤ 25 years ($p<0.01$) and age group > 25 years ($p<0.001$)] as compared to control. Significant change ($p<0.001$) in glucose and urea was also noted in case of tested population of female of both age group with respect to control of comparable age .

Figure 3

Table 2B: Effect of arsenic exposure on biochemical parameters in females

Biochemical parameters (Unit)	Age Group					
	Control (n=5)	Exposed (n=20)	Change (P value)	Control (n=18)	Exposed (n=61)	Change (P value)
	Age ≤ 25 years	Age ≤ 25 years		Age ≤ 25 years	Age >25 years	
Glucose (mg/dL)	81.28 \pm 2.84	76.47 \pm 2.42	0.001	79.71 \pm 2.35	86.01 \pm 2.96	0.001
Serum AST (U/L)	28.80 \pm 4.13	92.15 \pm 16.50	0.001	43.16 \pm 6.83	99.18 \pm 7.78	0.001
Serum ALT (U/L)	27.74 \pm 10.06	66.54 \pm 12.91	0.001	46.56 \pm 11.50	86.60 \pm 9.06	0.001
Serum ALKP (U/L)	58.00 \pm 17.78	213.05 \pm 28.88	0.001	87.33 \pm 25.35	145.63 \pm 6.09	0.001
Serum Total Protein (g/dL)	7.24 \pm 0.30	7.70 \pm 0.11	0.001	7.49 \pm 0.11	7.69 \pm 0.68	NS
Serum Albumin (g/dL)	4.92 \pm 0.16	4.77 \pm 0.08	0.01	4.95 \pm 0.05	4.75 \pm 0.04	0.001
Serum Urea (g/dL)	16.54 \pm 1.16	20.18 \pm 1.17	0.001	20.90 \pm 1.89	19.06 \pm 0.69	0.001

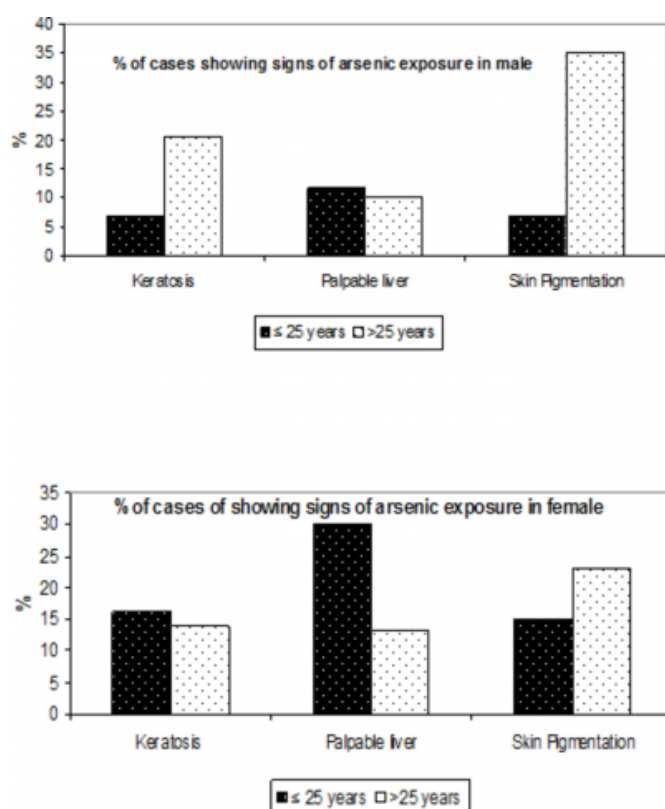
Values are mean \pm SD

Number in parenthesis indicating subjects

Clinical examination for detection of exposure induced palpability of liver, pigmentation, keratosis of the subjects is shown in figure 1. Arsenic induced palpability of liver was observed in female (30% cases) and male (11.70% cases) of age group ≤ 25 years. On the other hand, tested population of age group of >25 years showed liver palpability in 13% females and 10% males. It has been noted that 6.80 % male and 15% female of age group ≤ 25 years showed skin pigmentation whereas the corresponding figure was 35% in male and 23% in female for age group >25 years. Arsenic induced keratosis was observed in 6.80% male of age group ≤ 25 years and the rise in percentage of cases (20.7%) was noticed among subjects of age group >25 years. This trend was not seen among female.

Figure 4

Fig I Effect of arsenic on Clinical Manifestations



DISCUSSION

It has been observed that the biological indicators of hepatic effect parameters like AST, ALT, and ALKP rise as an effect of arsenic exposure in drinking water. Reports indicated that elevation was also observed as ALKP in 51.3%, ALT in 11.8% and AST in 27.6% cases with enlargement of liver in 76.9% cases (³). Other reports of elevation of liver enzymes also support our observation (⁷). The murine model, relevant to epidemic human toxicity in areas of arsenic concentration, also showed that continued arsenic feeding resulted in fatty liver with serum ALT, AST elevated at 12 months and hepatic fibrosis at 15 months. (⁵). Our studies also indicated that level of serum albumin, as biological indicator of liver biosynthetic capacity, declines although the effect of exposure on TP was not consistent with various age groups of tested population. Consequently, high level of serum globulin is expected. This observation is supported by other workers (¹⁸). Although blood glucose level was noted within the normal limit, significant elevation was observed in blood glucose of exposed subject (age group >25 years) in comparison to control. Reports are available on the normal level of blood glucose of arsenic affected patients. (¹⁹). Arsenic exposure is a risk factor for diabetes mellitus (²⁰). Increasing trends of plasma sugar with

indices of arsenic exposure in drinking water was found to be independent of the presence of skin lesions associated with arsenic exposure (²¹). The present study revealed the probability of exposed individuals to be diabetogenic. Level of serum urea was within the normal range but exposure dependent elevation was noticed in females compared to control. Other studies also showed that serum urea of arsenic affected patients was within normal range (⁷). The association between arsenic exposure and diabetes mellitus is a new finding (²¹). Our preliminary observation showed the elevation of glucose upon exposure. Studies in Bangladesh also supported our finding (²⁰). The present study showed some similarity of clinical manifestation including palpability of liver of both arsenic exposed males and females of tested population to the percentage of cases where elevated level of biomarkers were noticed. This observation was also supported by other workers (⁷).

All the studies so far carried out in different laboratories was involving patients and including effects of arsenic exposure in concentration dependent manner. To our knowledge, this is the first report where control subjects are included in the study to observe the effect of arsenic. Further studies are needed to identify and characterize the stress factors causing elevation of biomarkers induced by arsenic.

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