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Is An Elevated Antinuclear Antibody Titer In Subjects Living In Two Groundwater Arsenic Contaminated Villages Indicative Of A Time-Dependent Effect Of Arsenic Exposure

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ABSTRACT

The arsenic (As) contents of urine and blood and anti-nuclear antibody (ANA) titer in blood sera were determined in some 47 volunteers from Ghetugachhi and 83 from Dakshin Panchpota, two arsenic contaminated villages, and in 26 volunteers from a distant arsenic-free village, Padumbasan, in West Bengal. Hair and nail samples from some of them were also analyzed. While As content of urine, blood, hair and nail samples in control subjects from Padumbasan was in the non-detectable range and blood sera of all except one were ANA negative, many volunteers from the two arsenic contaminated villages had a high level of 'As' content in their urine, blood, hair and nail and were ANA positive. Many of the latter group had skin and digestive disorders. There was some positive correlation among arsenic content of blood, urine and hair. Although no clear-cut correlation between As content in different samples and ANA positivity could be substantiated, the high incidence of ANA could probably be due to long-term digestive disorders brought about by chronic arsenic intake. Test of ANA titers in arsenic risk zones may be useful as an additional parameter in risk assessment along with periodical monitoring of As in urine and nail samples.
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KEYWORDS

Autoimmunity;
Anti-nuclear antibody;
Arsenic toxicity;
Environmental health;
Groundwater contamination;
Systemic lupus erythematosus.

INTRODUCTION

One of the worst water related problems causing a great amount of harm to a large section of mankind in recent years is the contamination of drinking groundwater with various toxic metal compounds including arsenic. Groundwater arsenic has affected millions of people globally distributed over some twenty countries[1], including over 100 million people in West Bengal (India) and Bangladesh alone[2]. Prolonged exposure to arsenic ('As') leads to various ailments and dysfunctions of several vital organs like liver, kidney, lung etc. Apart from its toxic effects manifested on skin and other epidermal tissue[3]. Most of the affected people in general complain of muscle and joint pains and are highly depressed with various gastric problems. However, although much efforts have so far been directed towards detection and mobilization of arsenic in groundwater and soil, or towards recording the nature of various symptoms and diseases in people[4-6] of arsenic affected areas, no systematic survey on incidence of auto-immune disorder along with analysis of As content in different human samples living in high risk arsenic contaminated villages has been carried out earlier except for some case studies on ANA titers in patients with arsenicosis[7-8].

In the present communication, the environmental hypothesis being tested is whether chronic groundwater arsenic contamination has any correlation with elevation of antinuclear antibody noticed in people living in high risk arsenic villages in India.

EXPERIMENTAL

Study population

Arsenic had been detected in tube wells of the two villages, Ghetugachhi and Dakshin Panchpota under Chakdaha Block, District Nadia, West Bengal, first in 1995 and 2002, respectively, where the study was conducted. Arsenic-free water plants have been installed only in November 2002, and December 2004, respectively, in these two villages, from where the villagers mainly drink water, but generally use other water sources for cooking and other purposes. Therefore, although the villagers of Ghetugachhi had been exposed to groundwater arsenic contamination since 1995, they also had facilities of arsenic free drinking water since 2002, whereas the villagers of Dakshin Panchpota had been exposed to arsenic since 2002, but got facility of arsenic free drinking water only since December 2004.

A detailed structured interview was conducted of the volunteers of both affected and unaffected villagers spanning the residential and drinking water histories, dietary habits, life style etc. Which revealed that they had a fairly similar food habit and life style in these villages. The male villagers as well as unmarried women were permanent residents of the villages since birth, but the womenfolk married to the villagers became residents only after their marriage. 26 volunteers collected randomly from permanent residents of a distant village, Padumbasan (about 160 km away from district Nadia) in the district Purba Medinipur, where no groundwater arsenic contamination has so far been reported (and also confirmed by us), served as controls. Most of the volunteers had generally a good health albeit with minor gastric problem, but two of them complained of occasional muscle and joint pains. But 'As' was below detectable limit in the samples of all of them, for which their 'As' or ANA data have not been shown in the tables.

Along with the general symptoms of extreme fatigue and various digestive disorders, rheumatic joint and muscle pains, gastric and urinary problems were some of the common symptoms prevalent in villagers of the two arsenic contaminated villages. Quite a few people also had scleroderma and various skin diseases.

Determination of As content in urine, blood, nail, hair and nail samples

'As' content of first void morning urine and fasting blood of 47 randomly selected volunteers of different age-groups and sexes from the village Ghetugachhi (Gr-I), and 83 from Dakshin Panchpota village (Gr-II), as also 26 volunteers from Padumbasan village (negative control), and hair and nail samples of some subjects, was determined by a atomic absorption spectrophotometer adopting the standard AAS protocol[9-10]. Hair and nail samples could not

be collected from all volunteers, because of either lack of grown nail on the day of collection or else, because of their taboo or superstition against giving hair or nail samples. Arsenic content of different drinking water samples was also determined.

Their blood sera were subjected to ANA test by using an ANA Detect kit (ANA ORG 600; ORGENTEC Diagnostika GmbH, Germany) with the aid of an ELISA Reader (ELDEX 3.8, USA). This assay collectively detects, in one well, ANAs against some twenty antigens, including double stranded DNA (ds DNA, nDNA), histones, SS90 A/Ro, SS-B/La, Sm, SmRNP, Scl-70, PM-Scl-100, Jo-1, and centromeric antigens. Those who tested ANA positive, were also tested for Scl 70 (as some of the victims had characteristic skin symptoms), with specific kit procured from the same source (ORGENTEC Diagnostika GmbH, Germany). As ELISA test gives a better and dependable result for detection of ANA titer, this method was preferred.

Statistical analysis

The level of correlation significance was calculated by Pearson test (1-tailed) using SPSS software version 10.

RESULTS AD DISCUSSION

'As' content in water collected from different tube wells

The 'As' content of water from three tube wells of Padumbasan village tested was found to be below detectable limit. However, although the 'As' content of water collected from the arsenic-free plants of both Ghetugachhi and Dakshin Panchpota tested 'As' content below 10 ppb (within permissible range up to 20 ppb) on an average of three tests done on three different days, some other tube wells which were also being used for cooking and washing purposes in both the villages had water samples measuring an 'As' content between 65.9 ppb and 330.37 ppb in Ghetugachhi (10 tube wells tested) and between 94.35 ppb and 339.38 ppb in Dakshin Panchpota village (6 tube wells tested).

ANA titers and 'As' content in urine, blood, hair and nail

The 'As' contents in urine, blood, hair, nail and ANA titers of volunteers of two arsenic villages have been provided in TABLE 1.

Quite a few villagers in both the arsenic infested villages showed 'As' contents in their samples in higher quantities than the permissible normal limits.

A positive correlation among blood, hair, nail of group I ($p<0.01$ among blood, nail, and hair; TABLE 2) could be established if the blood arsenic level was compared with that of hair and nail by Pearson Correlation using SPSS software version 10. Similarly, a positive correlation ($p<0.05$ to 0.01) among blood, urine, hair and nail in Gr II subjects (TABLE 3) could also be obtained.

For a critical analysis of any possible relationship to exist between ANA titer to either age or sex, the data have been shown in a classified manner (TABLE 4). However, the data did not reveal any specific relationship per se, as all age-groups appeared to be vulnerable.

An analysis of the data to draw relationship with ANA positivity also could not reveal any specific relationship, because while some ANA positive subjects had a higher urinary or blood 'As' content, others had their 'As' content in the normal range. Some subjects, even those with normal 'As' range in both blood and urine showed their sera to be ANA positive. Alternatively, some subjects showing both urinary/blood 'As' range exceeding the higher limit, still had ANA negative titers. The same trend also appeared to be true in case of hair and nail samples (TABLES 5a,b).

When the 'As' contents of nail and hair were correlated to the occurrence of positive ANA titers, no correlation with either hair or nail 'As' content of groups I and II subjects (TABLES 6, 7) could be established. Interestingly, some subjects apparently did not show any external symptoms of arsenicosis, still they were ANA positive. Only one ANA positive subject tested Scl-70 positive.

An analysis of 'As' content in urine, blood, hair and nail would reveal that although villagers of Ghetugachhi had been drinking arsenic free water since 2002, some of them still show high levels of arsenic in their urine as well as blood. The same was

TABLE 1: Showing age, sex, As content in urine and blood and gross symptoms of subjects from two arsenic contaminated villages.

Sl. No. Gr. I	Age	Sex	ANA	As (Nail) (Normal range 0.43-1.08ppb)	As (hair) (Normal range 0.08-0.25ppm)	As (Blood) (Normal range 0.3-2.0 ppb)	As (Urine) (Normal range 3.33-25.33 ppb)	Sl. No. Gr. II	Age	Sex	ANA	As (Nail) (Normal range 0.43-1.08ppb)	As (hair) (Normal range 0.08-0.25ppm)	As (Blood) (Normal range 0.3-2.0 ppb)	As (Urine) (Normal range 3.33-25.33 ppb)
1	25	M	1.043[b]	0.491	0.342	ND	32.216	1	47	M	-	3.4	1.479	ND	46.225
2	22	M	1.325*	0.867	ND	36.470	15.950	2	50	M	0.887	ND	3.576	ND	ND
3	25	M	1.609*	0.867	ND	9.340	18.900	3	25	M	0.523	ND	3.576	ND	25.762
4	37	M	0.956	1.607	0.041	8.050	53.162	4	60	M	1.066[b]	ND	3.193	ND	134.712
5	45	F	1.542*	1.503	0.432	11.750	32.883	5	40	M	1.038[b]	ND	3.227	ND	ND
6	13	M	1.636*	0.837	0.196	ND	86.475	6	35	M	0.917	2.048	0.528	ND	ND
7	30	M	1.885*	0.926	0.181	ND	6.675	7	32	M	0.898	ND	1.422	ND	90.525
8	35	M	2.645*	0.328	0.012	ND	21.500	8	55	M	1.338*	ND	0.532	ND	17.787
9	34	M	1.879*	2.108	0.437	5.950	28.775	9	50	F	0.674	ND	2.476	0.750	18.400
10	19	M	3.812*	0.939	0.166	ND	33.937	10	41	F	0.768	ND	0.211	ND	45.487
11	25	F	1.545*	0.873	0.196	ND	19.925	11	30	F	0.898	11.995	1.529	ND	26.112
12	28	M	2.008*	1.526	0.43	5.450	22.112	12	50	M	-	5.72	0.266	27.95	99.225
13	34	M	1.876*	0.895	0.385	ND	39.937	13	50	F	1.407*	ND	-	ND	54.587
14	40	M	1.357*	0.478	ND	16.500	31.887	14	45	F	1.206*	ND	0.137	ND	1.975
15	42	M	1.563*	1.196	0.602	2.200	9.412	15	40	F	2.057*	ND	ND	ND	8.112
16	26	F	1.809*	0.834	0.603	ND	9.250	16	55	F	1.713*	ND	ND	ND	99.000
17	12	M	1.700*	1.086	0.39	ND	53.137	17	42	F	0.685	ND	1.242	17.750	42.925
18	42	M	1.181[b]	1.353	0.39	5.450	17.675	18	31	M	1.082[b]	ND	1.117	ND	139.412
19	35	F	1.841*	1.285	0.607	1.375	23.775	19	50	F	1.325*	0.14	0.224	ND	ND
20	10	M	1.847*	2.311	1.146	ND	63.850	20	32	M	1.146[b]	ND	ND	2.150	13.625
21	40	M	1.923*	1.74	0.518	ND	45.375	21	57	M	2.126*	ND	0.128	ND	32.050
22	17	M	1.322*	1.193	0.9	0.975	12.487	22	35	F	0.997	ND	ND	ND	ND
23	44	M	1.466*	ND	ND	ND	77.700	23	55	M	0.793	0.732	1.327	ND	1.800

***=ANA positive; [b]=borderline**

true for villagers of Dakshin Panchpota who had been using arsenic-free drinking water since December 2004. Therefore, apparently it takes quite a long period for arsenic to be deposited in nail and hair follicles and also quite a long period to be free of arsenic even after no fresh deposition or only little deposition takes place. However, nails and hair may not be always very reliable as strict indicators of internal arsenic deposition, as these are also subject to external arsenic contamination from other sources (e.g. bathing with contaminated water). Therefore, the arsenic levels in urine and blood should provide more dependable reflections of fresh contamination and therefore confirm that villagers in both arsenic affected villages still intake some amount of arsenic, presumably from other sources than water only, as they are provided with arsenic-free drinking water source. However, a few of those who showed arsenic in their urine or blood might also have consumed some water from the arsenic contaminated tube wells as well, particularly during their work in the field. Therefore, intake amount being unknown and to some extent variable in each volunteer, the absence or presence of urinary arsenic in levels higher

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TABLE 2: Showing pearson's correlations in group I subjects

		Blood	Urine	Hair	Nail
Blood	Pearson correlation	1.000	-0.199	-0.265	0.031
	Sig. (1-tailed)	0	0.093	0.095	0.440
	N	47	46	26	26
Urine	Pearson correlation	-0.199	1.000	0.155	0.160
	Sig. (1-tailed)	0.093	0	0.230	.223
	N	46	46	25	25
Hair	Pearson correlation	-0.265	0.155	1.000	0.602**
	Sig. (1-tailed)	0.095	0.230	0	0.001
	N	26	25	26	26
Nail	Pearson correlation	0.031	0.160	0.602**	1.000
	Sig. (1-tailed)	0.440	0.223	0.001	0
	N	26	25	26	26

**** Correlation is significant at the 0.01 level (1-tailed)**

TABLE 3: Showing pearson correlation in group II subjects

		Blood	Urine	Hair	Nail
Blood	Pearson correlation	1.000	0.183	-0.060	0.018
	Sig. (2-tailed)	0	0.097	0.722	0.941
	N	83	83	38	20
Urine	Pearson correlation	0.183	1.000	0.032	0.448*
	Sig. (2-tailed)	0.097	0	0.848	0.047
	N	83	83	38	20
Hair	Pearson correlation	-0.060	0.032	1.000	0.586**
	Sig. (2-tailed)	0.722	0.848	0	0.007
	N	38	38	38	20
Nail	Pearson correlation	0.018	0.448*	0.586**	1.000
	Sig. (2-tailed)	0.941	0.047	0.007	0
	N	20	20	20	20

*** Correlation is significant at the 0.05 level (2-tailed)**
**** Correlation is significant at the 0.01 level (2-tailed)**

TABLE 4: Classified data on expression of ANA in relation to age and sex. Total subjects studied 156, Padumbasan 26 (Male 23, Female 3), Ghetugachhi 47 (Male 33, Female 14), Dakshin Panchpota 83 (Male 50, Female 33) B=borderline

Name of villages	Padumbasan						Ghetugachhi						Dakshin panchpota					
Age group	Male			Female			Male			Female			Male			Female		
	+ve	B	-ve	+ve	B	-ve	+ve	B	-ve	+ve	B	-ve	+ve	B	-ve	+ve	B	-ve
<20 years	0	0	0	0	0	0	9	1	1	3	1	0	2	0	9	0	0	3
20-40 years	0	0	18	1	0	0	13	1	2	7	0	0	4	4	14	2	1	12
>40 years	0	0	5	0	0	2	5	1	0	3	0	0	4	1	12	4	1	10

TABLE 5a: Showing relationship of urinary and blood "As" content with that of ANA titer of blood sera

As content		ANA +ve		ANA -ve		ANA borderline	
		Male	Female	Male	Female	Male	Female
Above normal	Urine (>25.33 ppb)	26	8	30	21	5	3
	Blood (>2.0 ppb)	12	6	1	3	3	1
	Both Urine And Blood	3	2	6	4	1	1
Normal	Urine (<25.33 ppb)	13	11	5	4	3	0
	Blood (<2.0 ppb)	25	13	30	20	4	2
	Both Urine And Blood	10	8	5	4	1	0

ANA positive > 1.2; ANA borderline between 1.0 and 1.2; ANA negative < 1.0 (as per specification of kit)

than the normal limits may actually indicate more on how much arsenic they ingested the previous day or previous 4 days, as arsenic (particularly the organic forms) is known to stay for about ten hours in the blood and then may be excreted through urine for the next 96 hrs or so[11]. Further, urinary excretion also depends on the state of kidney functioning. This could possibly explain why a direct and valid correlation of ANA positivity to urinary or blood 'As' contents was not possible. But from the analysis of ANA data, it becomes clear that subjects from Ghetugachhi village show more number of ANA-positive cases than in Dakshin Panchpota village where the contamination has been noted in recent

TABLE 5b: Showing relationship of nail and hair "As" content with that of ANA titer of blood sera

As content		ANA +ve		ANA -ve		ANA Borderline	
		Male	Female	Male	Female	Male	Female
Above normal	Nail (>1.08ppb)	1	0	7	2	1	0
	Hair (>250ppb)	2	0	10	4	4	0
	Both Nail And Hair	1	0	5	1	1	0
Normal	Nail (<1.08ppb)	3	5	8	7	4	1
	Hair (<250ppb)	2	5	5	5	1	1
	Both Nail And Hair	2	5	3	4	1	1

ANA positive > 1.2; ANA borderline between 1.0 and 1.2; ANA negative < 1.0

TABLE 6: Showing pearson correlations in group I subjects

		Blood	Urine	Hair	Nail	Ana
Blood	Pearson correlation	1.000	-0.199	-0.265	0.031	-0.092
	Sig. (1-tailed)	0	0.093	0.095	0.440	0.270
	N	47	46	26	26	47
Urine	Pearson correlation	-0.199	1.000	0.155	0.160	-0.051
	Sig. (1-tailed)	0.093	0	0.230	0.223	0.369
	N	46	46	25	25	46
Hair	Pearson correlation	-0.265	0.155	1.000	0.602**	0.022
	Sig. (1-tailed)	0.095	0.230	0	0.001	0.458
	N	26	25	26	26	26
Nail	Pearson correlation	0.031	0.160	0.602**	1.000	-0.129
	Sig. (1-tailed)	0.440	0.223	0.001	0	0.265
	N	26	25	26	26	26
Ana	Pearson correlation	-0.092	-0.051	0.022	-0.129	1.000
	Sig. (1-tailed)	0.270	0.369	0.458	0.265	0
	N	47	46	26	26	47

TABLE 7: Showing pearson correlations in group II subjects

		Blood	Urine	Hair	Nail	Ana
Blood	Pearson correlation	1.000	0.183	-0.060	0.018	0.155
	Sig. (1-tailed)	0	0.049	0.361	0.471	0.081
	N	83	83	38	20	83
Urine	Pearson correlation	0.183	1.000	0.032	0.448**	-.234*
	Sig. (1-tailed)	0.049	0	0.424	0.024	0.017
	N	83	83	38	20	83
Hair	Pearson correlation	-0.060	0.032	1.000	0.586**	-0.067
	Sig. (1-tailed)	0.361	0.424	0	0.003	.345
	N	38	38	38	20	38
Nail	Pearson correlation	0.018	0.448**	0.586**	1.000	-0.199
	Sig. (1-tailed)	0.471	0.024	0.003	0	0.200
	N	20	20	20	20	20
Ana	Pearson correlation	0.155	-0.234*	-0.067	-0.199	1.000
	Sig. (1-tailed)	0.081	0.017	0.345	0.200	0
	N	83	83	38	20	83

** Correlation is significant at the 0.01 level (1-tailed)

past and arsenic free water plant has also been commissioned after two years of detection of groundwater contamination in this village. Thus, this may indicate that the exposure time to arsenic may be an important factor in elevating ANA titer, presumably by affecting the liver through its toxicity. It also points to the fact that once the ANA titer is elevated, it does not tend to revert back to its normal level even though the intake of fresh arsenic becomes drastically reduced. Further, as the subjects of both villages come from the same socio-economic background and mostly live on varying undernourished diet, the recovery seems to be delayed.

Some implications of occurrence of ANA positive titers and autoimmune disorders in public health

The Ghetugachhi population had an alarmingly high frequency of occurrence of ANA in their blood sera. Out of 47 subjects, some 40 (85.1%) tested positive and 4 others were in the borderline (TABLE 3). In Dakshin Panchpota, a total of 16 persons (19.2%) out of 83 tested positive while 7 were in the threshold. Whether the occurrence of very high incidence of ANA was due to the liver disorders found in majority of the subjects, particularly in the village Ghetughachhi, could not be firmly suggested although such a possibility could not be totally ruled

out, because ANA's are reported to be present in lower titers in several disorders that include liver diseases, leprosy, multiple sclerosis, juvenile rheumatoid arthritis, etc[12].

The development of autoimmune disorder in such a large scale as a consequence of chronic arsenic poisoning is a cause for great public health concern, particularly in the third world countries where awareness is rather limited. The occurrence of ANA positive titers is detrimental to health and can lead to various fatal diseases. In fact, already large-scale premature mortality has been reported in these villages. In this initial survey, people of almost all age-groups were found to be vulnerable; males were more vulnerable than females. Incidentally, in normal random populations, the incidence of ANA positive cases is reported to be only about 5%, generally much higher in females (9 female : 1 male)[12], and particularly in women during their child-bearing years. But clearly, the present study showed that the males are more vulnerable in the arsenic prone areas, and there was no indication that age was a factor in being ANA positive.

CONCLUSION

Antinuclear antibodies are diverse groups of autoantibodies that are generally found in systemic autoimmune disorders like systemic lupus erythomatosus (SLE), Sjogren's syndrome (SS), systemic sclerosis (SSc), inflammatory myositis (IM), mixed connective tissue disorder (MCTD) and rheumatoid arthritis (RA). The present study suggests that chronic exposure to groundwater arsenic can also be a cause for elevation of ANA titers in human living in high risk arsenic areas. Therefore, the detection of antinuclear antibodies in high risk arsenic zones can also be fruitfully utilized as an indicator or biomarker in early risk assessment or for ascertaining extent of damage to internal organs in affected people, apart from its normal use in aiding the diagnosis in several autoimmune disorders, because, quite a few villagers in both villages were apparently not yet showing up symptoms of any inflammatory rheumatic diseases, or of arsenicosis, yet they tested ANA positive.

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