# Role of Folic Acid on Symptoms of Chronic Arsenic Toxicity

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## ABSTRACT

**Background:** Chronic arsenic toxicity (Arsenicosis) due to drinking of arsenic contaminated ground water is a global problem. However, its treatment is unsatisfactory. Methylation of arsenic facilitates its urinary excretion. Persons with relatively lower proportion of urinary dimethyl arsenic acid (DMA) are found to have at greater risk of developing symptoms of arsenicosis including its complications. The biochemical pathway responsible for methylation of arsenic is a folate-dependent pathway. Studies in rodents and humans suggest that folate nutritional status influences the metabolism of arsenic.

**Methods:** The present study compares the effect of giving folic acid on 32 arsenicosis patients during a 6-month period and comparing the results with clinical effect of taking only arsenic-free safe water on 45 age and sex-matched arsenic-affected people for the same period.

**Results:** There was significant improvement of arsenical skin lesion score of both patients treated with folic acid (2.96 ± 1.46 to 1.90 ± 0.90, $P < 0.001$) and arsenic free safe water (2.91 ± 1.26 to 1.62 ± 1.05, $P < 0.001$) for a period of 6 months. Significant improvement in systemic disease score was also observed from the baseline systemic score in folic acid treated group (4.78 ± 3.43 to 1.00 ± 1.56, $P < 0.001$) and the group treated with arsenic-free water (1.87 ± 2.11 to 0.82 ± 1.62, $P < 0.001$).However, there was a significant increased improvement of systematic disease score in the folic acid treated group compared to the control group taking arsenic free water ($P < 0.001$).

**Conclusions:** This study provides evidence that folic acid treatment in arsenicosis cases could help in reducing clinical symptoms of arsenicosis.

**Keywords:** Arsenicosis, folic acid, nutritional deficiency, treatment of arsenicosis

Original Article

## INTRODUCTION

Arsenic contamination of groundwater has been recognized as a great threat to water supply and public health in many countries in the world. Pigmentation and keratosis are the specific

skin lesions characteristic of chronic arsenic toxicity (Arsenicosis). However, it also produces various systemic manifestations, common being chronic lung disease, polyneuropathy, liver fibrosis weakness, non-pitting edema of legs, anemia, and cancer of skin.

Chelation therapy for chronic arsenic toxicity is thought to be the specific therapy for relief of systemic clinical manifestations and reduction of arsenic stores in the body, reducing subsequent cancer risk. Piamphongsant[1] reported efficacy of D-penicillamine in the management of chronic arsenic toxicity. However, rain drop pigmentation and white macules remained unchanged in spite of therapy. Therapy with dimercapto succinic acid (DMSA) did not cause any significant clinical improvement compared to patients treated with placebo.[2] Therapy with dimercapto propane succinate (DMPS) caused significant improvement in the clinical condition of chronic arsenicosis patients as evidenced by significant reduction of total clinical scores compared to placebo. The most significant improvement was noted in regard to the clinical scores of weakness, pigmentation and lung disease.[3] However, the drug is a costly one, unsuitable for use of large number of poor arsenicosis patients living distant villages of many developing countries. Ahmad *et al.*, (1998) evaluated the effectiveness of management of chronic arsenicosis in Bangladesh by administering vitamin A, E, C regimen.[4] Improvement of melanosis and keratosis were observed in 90.9% and 86.4% of patients, respectively, from among 22 patients who had used safe water and had taken the regimen regularly. However, the characteristics of skin lesions for evaluation of severity of arsenicosis were not described, nor comparison of effect of placebo and use of arsenic free water were considered in the trial. Drinking predominantly arsenic free water increased the probability of regression in subjects with mild stage lesions but not in those with more advanced stage lesions. Guha Mazumder *et al.*, in a study conducted in arsenic endemic area of West Bengal, found that out of 199 people with skin lesion among the arsenic exposed population who were consuming safe water during the previous 5 years, the skin lesions cleared or decreased in 49.7% of people. However, out of 306 people who did not have such lesions previously, new skin lesions appeared in 32 (10.5%).[5] Oshikawa *et al.*,[6] investigated the changes of severity of skin lesions over a period of 10 years among an affected cohort in an area having arsenic contaminated shallow wells due to tin mining activities in Southern Thailand where interventions to reduce arsenic contaminated water had been implemented. Over 10 year period, both regression and progression of lesions occurred, though the majority of the subjects followed up remained the same.

Methylation of arsenic facilitates its urinary excretion. Persons with relatively lower proportion of urinary dimethyl arsenic acid (DMA) were found to have at greater risk of skin and bladder cancer[7-10] and peripheral vascular disease in Taiwan.[11,12] For this reason, methylation of Inorganic As (InAs) has traditionally been considered as detoxification pathway, however, Methyl Arsenic Acid III (MMA (III)) is more toxic than Inorganic As. Hepatic methylation of Inorganic As (III), which is highly variable in humans,[13] first generates monomethylarsonic acid (MMA (V)) and then reduced to monomethyl arsenous acid (MMA (III)). After reduction to MMA (III), a second methylation occurs to generate dimethylarsenic acid (DMA (V)). The biochemical pathway responsible for methylation of arsenic is a folate dependent pathway [Figure 1]. Studies in rodents and humans suggest that folate nutritional status influence the metabolism of arsenic.[12,14-19]

The present study describes an open trial with folic acid on a arsenicosis affected population and comparing the results with another population without the drug, both taking arsenic-free water and no other intervention during a 6-month period.

## METHODS

### Study subjects

Participants selected for administration of folic acid were recruited from two Arsenic clinics run by arsenic experts of DNGM Research Foundation (DN Guha Mazumder Research Foundation) at two State Government hospitals one at Baruipur Sub divisional hospital in South 24 parganas district and another at Ashoke Nagar Rural Hospital in North 24 Parganas district of State of West Bengal, in India, situated 30 and 45 km, respectively, away from Kolkata. Participants who attend the clinics suffer from symptoms of arsenic

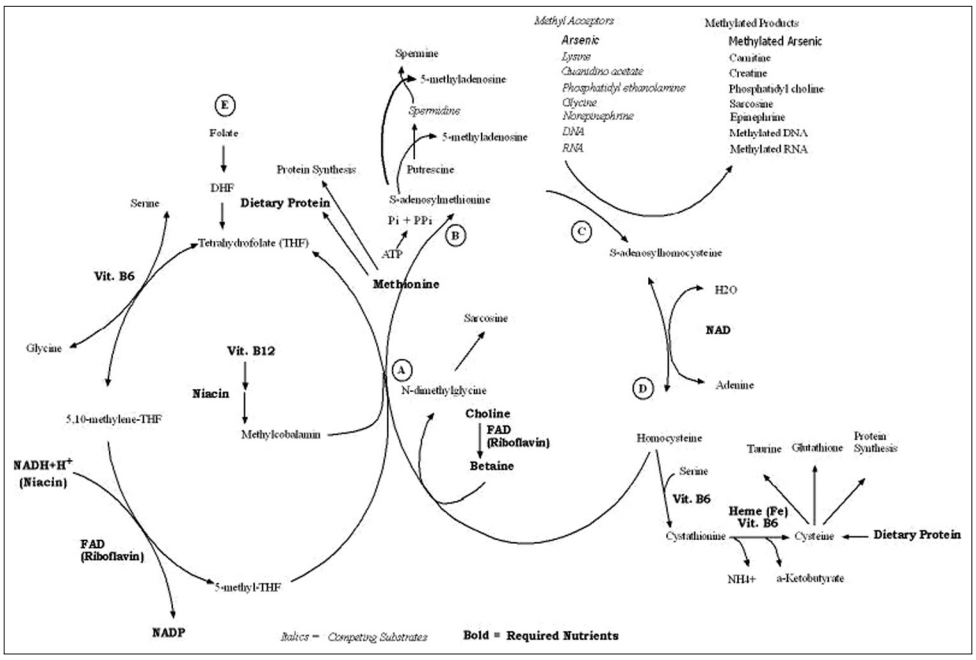


**Figure 1:** S-adenosylmethionine-linked metabolism (adapted from Donohue and Abernathy, 2001)

toxicity, live in neighboring villages and have history of drinking arsenic contaminated ground water. Participants having signs and symptoms of arsenic toxicity [diagnosed on the basis of world health organization 2005 (WHO 2005) criteria of diagnosis of clinically confirmed case of arsenicosis] who had recently switched over to arsenic free safe water source and agreed to attend the clinic every month and agreed to take the drug folic acid regularly for 6 months were included in the drug trial. Out of these 55 patients, who were enrolled initially, only 32 patients attended the clinic and took the drug regularly for 6 months and constituted the study subjects. As very few participants with signs and symptoms of arsenic toxicity attending the arsenic clinics agreed to be treated with placebo drug, control group could not be included from the clinic patients.

### Control subjects

The control population consisted of 45 patients from Raninagar II, Hariharpara, Domkal, Bhagabangola I, and Lalgola blocks of Murshidabad district in the State of West Bengal, situated about 200-250 km away from Kolkata. Control subjects were recruited from the area where the arsenic affected people were using filtered water through arsenic removal plants (ARP) installed by Pal Trockner and Co, part of a project by GTZ and Harbauer GmbH, Germany, who had taken up the task of providing arsenic free water to the rural population of the arsenic affected districts of West Bengal. To monitor the health effects of providing arsenic free safe water, base line, and biannual health checkups were done by arsenic experts of the Foundation. To ensure definite intake of arsenic safe water by the control group, a separate region, which was fully supplied byarsenics-free water by arsenic removal filters, was selected for ensuring assessment of efficacy of arsenic-safe water in reducing the symptoms of arsenicosis cases. Out of 123 arsenicosis cases (diagnosed on the basis of WHO criteria of diagnosis of clinically confirmed case of arsenicosis), who were drinking arsenic free water from the ARPs and were initially included in the study, 45 cases were found to be taking arsenic free water through ARPs regularly for 6 months and these cases were included as control subjects. This study was carried out by the same arsenic expert doctors of the Foundation who had been attending arsenic clinics at Baruipur and Ashoke Nagar hospitals and carried out the folic acid study.

### Inclusion criteria for patients

To be eligible, adults above the age of 18 years, both males and females with history of taking arsenic contaminated water but currently taking

**Table 1:** Dermatological criteria and gradation of chronic arsenic toxicity scoring system

| Mild (1) | Moderate (2) | Severe (3) |
|---|---|---|
| Pigmentation (Score) | | |
| Diffuse melanosis, Mild spotty pigmentation, Leucomelanosis | Moderate spotty pigmentation | Blotchy pigmentation, Pigmentation of under surface of tongue, Buccalmucosa |
| Keratosis (Score) | | |
| Slight thickening, or minute papules (<2 mm) in palm and soles | Multiple raised keratosis papules (2 to 5 mm) in palm and soles with diffuse thickening | Diffuse severe thickening, large discreet or confluent keratotic elevations (>5 mm), palm and soles (also dorsum of extremity and trunk) |

Maximum total skin score=6

safe water and having symptoms and signs of arsenicosis, determined by characteristic skin lesions of melanosis and keratosis and fulfilling WHO diagnostic criteria of clinically confirmed case of arsenicosis were included as participants belonging to both study and control group. Participants who agreed to give written consent to undergo the trial were only included in the study.

### Exclusion criteria for patients

- All patients not exposed to arsenic and without any clinical features of arsenicosis
- Patients having any concurrent illness due to other causes, known other skin disease or other chronic illness
- Patients known to have received any vitamins and minerals from local doctors
- Patients refusing to give consent.

Each selected participant was questioned briefly about his or her sources of drinking and cooking water and duration of water use from the source. Water collected from current and previous sources were tested for arsenic by Atomic absorption spectrophotometer with hydride generation system. After taking medical history from the participant general medical examination was carried out, including a careful inspection for arsenical skin lesions. Demographic characteristics and socio economic condition of the participant were also recorded in a proforma as a part of baseline survey.

The study group was given a tablet of 5 mg of folic acid daily and the control group continued to take arsenic-free safe water for 6-month period. Monthly checkup and replenishment of drug was carried out to folic acid study group. As a part of follow up survey, both the groups were clinically examined at the end of six month period and an objective scoring system was followed to evaluate the clinical outcome with and without drug administration.

The patients were evaluated by an objective scoring system [Table 1] before and after treatment. Skin scoring and systemic scoring were done as per standardized protocol described earlier.[2,20] Briefly, though many symptomatic parameters recorded were subjective, the objective parameters included were pigmentation, keratosis, chest signs (rales and rhonchi), hepatomegaly, and splenomegaly. Flushing of face, solid edema of legs and hands, ascites and absence deep reflexes for neuropathy were also included in the scoring system. Breathlessness at accustomed exertion, mild exertion, or at rest was defined as mild (1), moderate (2), and severe (3), respectively. Skin scoring was done based on mild, moderate and severe lesion of pigmentation and keratosis.[20]

After 6-months, the findings of skin and systemic score were compared with baseline skin and systemic score of the study and control group. Ethical committee of the Foundation, fulfilling the Helsinki's criteria and recommendation of Indian Council of Medical Research, Govt. of India, approved the study protocol.

### Statistical analysis

Data are reported as means±S.D. Statistical significance between groups was determined by analysis of variance with significance set at $P < 0.05$.

## RESULTS

There was no difference in mean age, sex, and body mass index (BMI) of the study group (people treated with folic acid) and control group (people taking arsenic safe water only) [Table 2]. However, the study group had past history of drinking water with higher level of arsenic (mean arsenic

level of 1.42 ± 1.41 mg/L) compared to control group (mean arsenic level 0.14 ± 0.13 mg/L, $P < 0.01$). The mean duration of arsenic intake was also longer (28.33 ± 12.84 yrs,) in the study group compared to control group (12.50 ± 13.65 yrs, $P < 0.0.001$) [Table 2]. Male participants constituted 56.25% and 68.09% among the study and control group, respectively [Table 2].

There was no significant difference in skin score between the study group (2.96 ± 1.46) and the control group (2.91 ± 1.26, $P > 0.8$). Significant improvement in mean skin score was observed in participants treated with folic acid (2.96 ± 1.46 to 1.90 ± 0.90, $P < 0.001$) and without it (2.91 ± 1.26 to 1.62 ± 1.05, $P < 0.001$) for a period of 6 months [Figure 2a and b]. However, the differences in improvement from baseline skin score and score after 6 months of observations with (1.06 ± 0.56) and without (1.29 ± 0.21) folic acid treatment were not found to be significantly different ($P > 0.005$). However, the differences in baseline skin score and score after 6 months of observations for both the groups were considered to ascertain whether folic acid administration had any beneficial effect over those who were only taking water with arsenic level less than 0.05 mg/L. There was significantly more reduction of skin score in the participants treated with folic acid than those treated without folic acid and this was found to be statistically significant ($P < 0.05$).

There was significant difference in baseline systemic disease score between patients belonging to study group (4.78 ± 3.43) and control group (1.87 ± 2.11, $P < 0.001$).There was a significant improvement of systemic score, (from 4.78 ± 3.43 to 1.00 ± 1.56) after 6 months treatment with folic acid ($P < 0.001$). In the control group also mean systemic score changed

**Table 2:** Comparison of data on treatment given with and without folic acid on arsenicosis patients

| | **With folic acid (*n*=32)** | | **Without folic acid (*n*=45)** | | ***P* value** |
|---|---|---|---|---|---|
| Mean±S.D. | | | | | |
| Age | 47.84±14.29 | | 44.58±9.61 | | 0.359 |
| BMI | 21.22±3.32 | | 20.18±3.10 | | 0.317 |
| Arsenic in water (mg/L) | 1.42±1.41 | | 0.14±0.13 | | 0.009 |
| Duration of water intake (yrs.) | 28.33±12.84 | | 12.50±13.65 | | 0.000 |
| | ***n*** | **%** | ***n*** | **%** | |
| Sex distribution | | | | | |
| Male | 18 | 56.25 | 30 | 68.09 | 0.354 |
| Female | 14 | 43.75 | 15 | 31.91 | 0.354 |
| Arsenic skin score | | | | | |
| Baseline skin score | | | | | |
| <1 | 0 | 0.00 | 0 | 0.00 | |
| ≥1-≤2 | 17 | 53.13 | 20 | 44.44 | 0.451 |
| >2-≤4 | 10 | 31.25 | 20 | 44.44 | 0.232 |
| >4-≤6 | 5 | 15.63 | 5 | 11.11 | 0.570 |
| After 6 months treatment | | | | | |
| <1 | 12 | 37.50 | 7 | 15.56 | 0.030 |
| ≥1-≤2 | 18 | 56.25 | 29 | 64.44 | 0.469 |
| >2-≤4 | 2 | 6.25 | 9 | 20.00 | 0.061 |
| >4 to ≤6 | 0 | 0 | 0 | 00.00 | |
| | **Mean±S.D.** | | **Mean±S.D.** | | |
| Baseline skin score | 2.96±1.46 | | 2.91±1.26 | | 0.873 |
| After 6 months treatment | 1.90±0.90 | | 1.62±1.05 | | 0.023 |
| Improvement of score after treatment | 1.06±0.56 | | 1.29±0.21 | | >0.005 |
| Systemic score | | | | | |
| Baseline total systemic score | 4.78±3.43 | | 1.87±2.11 | | 0.000 |
| Systemic score after 6 months treatment | 1.00±1.56 | | 0.82±1.62 | | 0.627 |
| Improvement of score after treatment | 3.78±1.87 | | 1.05±0.49 | | <0.001 |

BMI: Body mass index

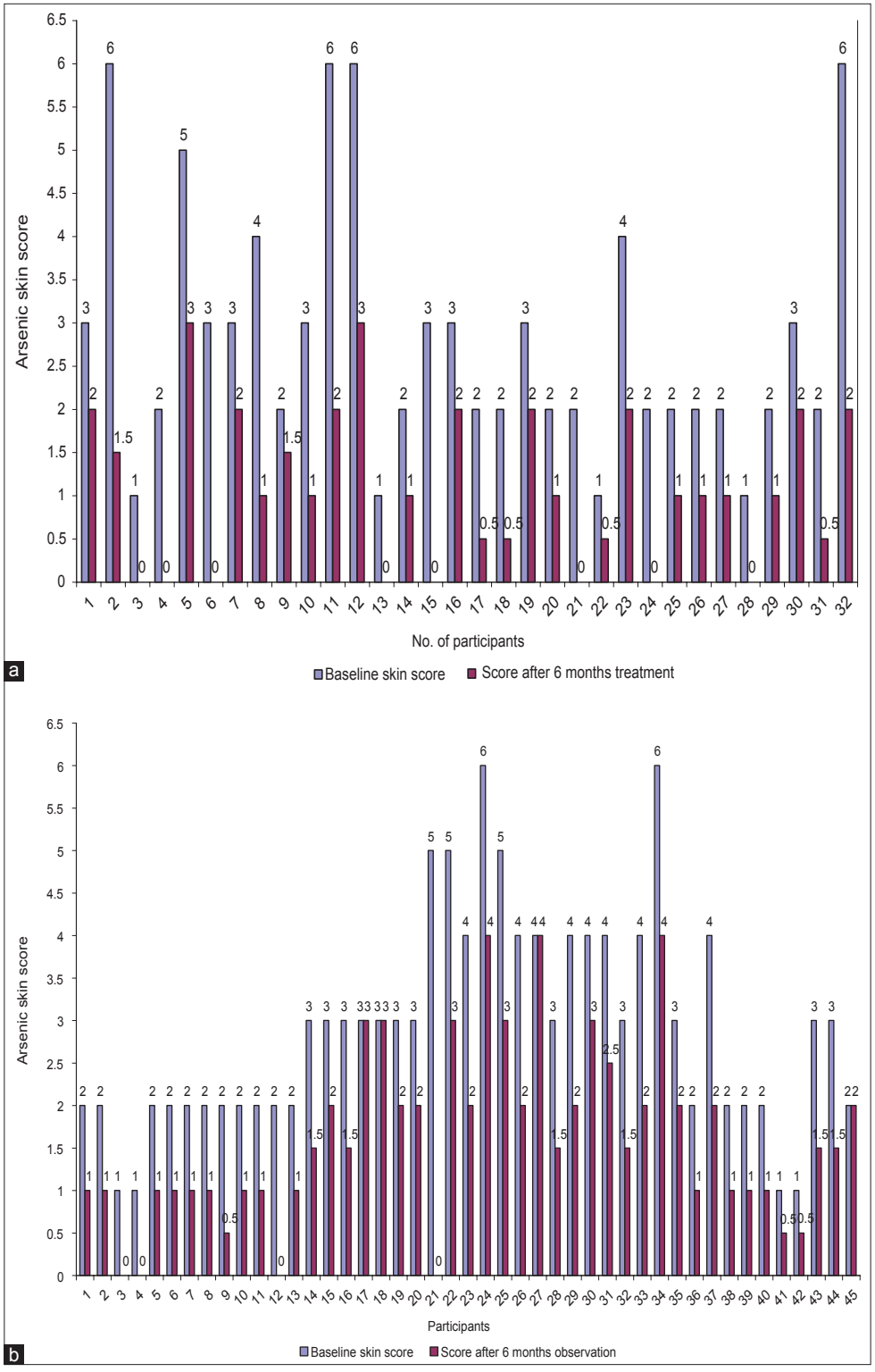


**Figure 2:** (a) Comparison of skin score of arsenicosis patients before and after treatment with folic acid. (b) Comparison of skin score of arsenicosis patients before and after taking arsenic free water for six months

from $1.87 \pm 2.11$ to $0.82 \pm 1.62$ with those taking only arsenic safe water for 6 months and this difference was statistically significant ($P < 0.001$). However, there was significantly more reduction of systemic disease score in the former group compared to the later ($P < 0.001$).

There was a significant improvement of systematic score after treatment with folic acid from baseline score (3.78 ± 1.87) in comparison to systematic score without folic acid (1.05 ± 0.49) and this improvement in reduction was statistically significant with $P < 0.001$.

## DISCUSSION

In an earlier study conducted in a rural district of South 24-Parganas of West Bengal, it was observed that low intake of folate in association with low animal protein, calcium, fiber, and vitamin C in diet may increase the risk of arsenic induced skin lesions.[18] Further, in a doubled-blind, placebo-controlled folic acid supplementation trials in Bangladesh, it was found that folic acid supplementation to participants with low plasma folate enhances arsenic methylation and reduces arsenic related health problem.[12] In a similar cross sectional study in Bangladesh, it was found that folic acid along with B group of vitamins and antioxidants modify the risk of arsenic related skin lesions.[19] However, this is the first study showing efficacy of Folic acid given for a period of 6 months causes improvement of clinical symptoms of arsenicosis compared to drinking of arsenic free water for the same duration.

In this study, improvement of systemic disease symptom score was found to be significant in folic acid treated group compared to those taking arsenic safe water while improvement in skin score was not significantly different between the two groups. In an earlier study Guha Mazumder *et al.*, (2001)[3] reported the efficacy of treatment of dimercapto propane succinate (DMPS), a chelating agent, in a single blind placebo controlled trial in patients suffering from chronic arsenic toxicity in West Bengal. Therapy with DMPS caused significant improvement in the clinical condition of chronic arsenicosis patients as evidenced by significant reduction of total clinical scores. Exposure cessation alone with placebo treatment also reduced clinical scores, but the post treatment total clinical score of DMPS-treated patients was significantly lower than that of placebo treated patients. The most significant improvement was noted in regard to the clinical scores of weakness and lung disease. No difference was noted between groups in regard to skin lesion like keratosis and skin histology before and after treatment.

Metabolism of InAs occurs in the body, predominantly by hepatic methylation, generating in sequence MMA (V), MMA (III), and DMA (V).[14,21] Methylation facilitates the urinary excretion of arsenic[22] and pentavalent methylated arsenic is less reactive than InAs.[23] Study in folate-deficient arsenic exposed people in Bangladesh after supplementation of folic acid for some period showed increase in the proportion of total urinary arsenic excretion as DMA in the folic acid group compared to the placebo group as was the reduction in proportion of total urinary arsenic excreted as MMA and as InAs.[19] The data indicated that folic acid supplementation to participants with low plasma folate enhances arsenic methylation. Increased methylation of arsenic by folate is hypothesized on the premise that arsenic is methylated by folate-dependent one-carbon metabolism with the use of S-adenosylmethionine (SAM) as the universal methyl donor.[21] Methionine biosynthesis in the methionine synthase reaction utilizes 5-methyl-tetrahydrfolate as a co-substrate and cobalamine as a cofactor in the remethylation of homocysteine.,[24] Subsequently, methionine is activated by Adenosine-5'-triphosphate (ATP) to generate SAM., SAM-dependent methylation reaction yields the methylated product (in case of arsenic MMA, DMA) and S-adenosylhomocysteine (SAH). Hydrolysis of SAH generates adenosine and homocysteine, but this reaction is readily reversible. As a consequence, plasma SAH concentrations increase linearly with even mild elevation in concentrations of homocysteine.[25] SAH is a potent inhibitor of most transmeythylation reactions,[25] including those of arsenic.[26] SAH binds tightly to methyltransferases and is removed only if the pathway is pulled forward by downstream removal of homocysteine, as may be achieved with folic acid supplementation.[19] Gamble *et al.*,(2006) showed in their well-controlled study that folic acid supplementation to participants with low plasma folate enhances arsenic methylation. Because persons whose urine contains low proportions of DMA and high proportions of MMA and InAs have been reported to be at greater risk of skin and bladder cancers and peripheral vascular disease, these

authors suggested that folic acid supplementation may reduce the risk of arsenic-related health outcomes.[27] However, our study showed evidences of significant improvement of arsenical symptoms in arsenic exposed people treated with folic acid irrespective of their nutritional status.

In the folic acid supplementation trials in Bangladesh,[19] enhanced arsenic excretion in urine as DMA associated with folic acid supplementation to participants with low plasma folate leading to reduced arsenic related health problem could be explained by the fact that about 55% of the participants were still drinking arsenic contaminated water during the study, and hence, they needed increased methylation for detoxification of continued exposure and increased excretion of DMA in urine. However, in the current study all the participants treated with and without folic acid were getting arsenic free water throughout the 6 months period of study. Hence increased symptomatic improvement with folic acid in the treated group may be difficult to explain. But, though the folic acid treated group were using arsenic free water for drinking and cooking purposes, they had still some increased arsenic exposure through diet as rice, the staple food of participants living in arsenic endemic areas was reported to contain high arsenic,[28,29] and many would be inadvertently taking arsenic contaminated water from their work places. We have also observed increased arsenic excretion in urine in a cohort population in Nadia, West Bengal taking arsenic free water and they had evidence of high arsenic intake in their diet.[30] Thus, in an arsenic endemic region one cannot prevent some arsenic exposure in spite of stopping arsenic free water supply in the household. Oshikawa *et al*., (2001) investigated the changes of severity of skin lesions over a period of 10 years among an affected cohort in an area having arsenic contaminated shallow wells due to tin mining activities in Southern Thailand where interventions to reduce arsenic contaminated water had been implemented.[6] Over 10 year period, both regression and progression of lesions occurred, though the majority of the subjects followed up remained the same. Drinking predominantly arsenic free water increased the probability of regression in subjects with mild stage lesions but not in those with more advanced stage lesions. By contrast, high arsenic content in the household well water, even though it was not used for drinking, decreased the probability of lesion regression among the subjects in more advanced stage but not among milder stage cases. Irrespective of initial stage a period of absence from the affected area increased the likelihood of lesion regression.

The limitation of the study was that this was an open trial with one group receiving folic acids while the control group taking arsenic safe water. Trial with double blind fashion could not be done because of logistic reason as the trial was conducted in distant villages far away from the city of Kolkata. Further, the study and control subjects did not live in the same geographical location nor did they have similar degree of arsenic exposure. The folic acid study group was exposed to higher dose and duration of arsenic exposure than the control group. This was due to the fact that the arsenic clinic patients had more systemic symptoms (Mean symptom score: 4.78 ± 3.43) motivating them to seek medical attention in hospital than systemic symptom score of population based study of control cases (Mean symptom score: 1.00 ± 1.56). However, though the folic acid study group had higher dose and duration of arsenic exposure and higher mean systemic score, results of 6 months treatment with folic acid in this group had higher degree of symptomatic improvement compared to control group. It was difficult to explain no significant difference in mean dermatological score between study cases and control subjects in spite of significant difference in dose and duration of arsenic exposure between the two groups. However, variation in dermatological manifestations was found in studies carried in West Bengal in spite variation of doses of arsenic exposure in different district studied. In a study, on a population of 7,683 in South 24 Parganas, prevalence of arsenical skin manifestation was found to be 8.8% and prevalence of neuropathy was found to be 4.7% with arsenic contamination in drinking water varying from 50 to 3,400 μg/L (Guha Mazumder *et al*., 2003).[31] On the other hand, in another study carried out in Nadia, out of 10,469 participants examined, 15.43% patients showed clinical features of arsenical skin lesion, and neuropathy was found to be 15.9%, the highest arsenic contamination in drinking water found being 1,362 μg/L.[20]

## CONCLUSIONS

This study provides evidence that folic acid treatment in arsenicosis cases could help in reducing clinical symptoms of arsenicosis.

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