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**Chapter 4 : Diagnosis and treatment of
chronic arsenic poisoning**

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Contents:

4. Diagnosis and treatment of chronic arsenic poisoning.	2
4.1. Diagnosis.	2
4.2. Biomarkers with special focus exclusively on diagnosis.	11
4.2.1 Urine.	12
4.2.2 Hair and Nail	16
4.2.3 Blood	18
4.2.4 Other Tissues	19
4.3. Treatment of chronic arsenic toxicity.	20
4.3.1 Chelators	21
4.3.1.1 DMSA (meso - 2,3- dimercaptosuccine acid, Succimer, Chemet) and DMPS (sodium 2,3 – dimercapto-1-propane sulfonic acid, Dimaval).	21
4.3.1.2 D-Penicillamine	26
4.3.2. Retinoids	27
4.3.3 Supportive and symptomatic treatment	28
4.4. Natural history	29
4.5. Outstanding questions and future research needs.	30
4.6. Executive Summary	34

4. Diagnosis and treatment of chronic arsenic poisoning

Humans are exposed to arsenic (As) primarily from air, food and water. However, elevated inorganic As in drinking water is the major cause of As toxicity. Most of the reports of chronic As toxicity in man focus attention on skin manifestations because of its diagnostic specificity, but As often affects most systems of the body. The clinical manifestations of chronic As intoxication are dependent on host susceptibility, the dose and the time course of exposure. The symptoms are often insidious in onset and varied in nature. However in a few epidemiological studies no significant clinical features of toxicity were attributed to chronic intake of As contaminated water.

4.1. Diagnosis

Although chronic As toxicity produces varied non malignant manifestations as well as cancer of skin and different internal organs, dermal manifestations such as hyperpigmentation and hyperkeratosis are diagnostic of chronic arsenicosis. The pigmentation of chronic As poisoning commonly appears in a finely freckled, “raindrop” pattern of pigmentation or depigmentation that is particularly pronounced on the trunk and extremities and has a bilateral symmetrical distribution (Fig. 4.1.1 - Mild pigmentation (a) Diffuse melanosis (with mild keratosis), (b) Mild spotty pigmentations, (c) Mild spotty depigmentations. (Guha Mazumder DN & Ghosh AK, personal collection) and Fig. 4.1.2 - (a) Moderate pigmentation, (b) Severe pigmentation. (Guha Mazumder DN & Ghosh AK, personal collection) (PENDING)) Pigmentation may sometimes be blotchy and involve mucous membranes such as the undersurface of the tongue or buccal mucosa (Black 1967; Yeh 1973; Tay 1974; Saha 1984, 1995, Guha Mazumder 1988, 1998a). The raindrop appearance results from the presence of numerous rounded hyperpigmented or hypopigmented macules (typically 2-4 mm in diameter) widely dispersed against a tan-to-brown hyperpigmented background (Tay 1974). Although less common, other patterns include diffuse hyperpigmentation (melanosis) (Tay 1974; Saha 1984), and localized or patchy pigmentation, particularly affecting skin folds (Tay 1974; Szuler et al. 1979; Luchtrath 1983). So-called leukoderma or leukomelanosis, (Saha 1984, 1995) in which the hypopigmented macules take a spotty, white appearance usually occur in the early stages of intoxication.

Arsenical hyperkeratosis appears predominantly on the palms and the plantar aspect of the feet, although involvement of the dorsum of the extremities and the trunk have also been described. In

the early stages, the involved skin might have an indurated, gritlike character that can be best appreciated by palpation; however, the lesions usually advance to form raised, punctated, 2-4 mm wartlike keratosis that are readily visible (Tay 1974). Occasional lesions might be larger (0.5 to 1 cm) and have a nodular or horny appearance occurring in the palm or dorsum of the feet. In severe cases, the hands and soles present with diffuse verrucous lesions (Fig. 4.1.3 (a) Mild keratosis, (b) moderate keratosis (i) moderate diffuse thickening of the palm, (ii) a few nodules over thickened palm (associated lesions : Bowen's disease of the abdomen and squamous cell carcinoma on the finger). (Guha Mazumder DN & Ghosh AK, personal collection) and Fig. 4.1.4 Severe keratosis (a) Verrucous lesion of the palm with keratotic horn, (b) Big nodules over the dorsum of feet (associated lesion : Squamous cell carcinoma). (Guha Mazumder et al, 1997) (PENDING)) . Cracks and fissures may be severe in the soles (Saha 1984, Guha Mazumder et al 1997). Histological examination of the lesions typically reveals hyperkeratosis with or without parakeratosis, acanthosis, and enlargement of the rete ridges. In some cases, there might be evidence of cellular atypia, mitotic figures, in large vacuolated epidermal cells (Black 1967; Tay 1974; Ratnam et al. 1992; Alain et al. 1993, Guha Mazumder et al., 1998c). Yeh (1973) classified arsenical keratosis into two types: a benign type A, further subgrouped into those with no cell atypia and those with mild cellular atypia; and a malignant type B, consisting of lesions of Bowen's disease (intraepithelial carcinoma, or carcinoma in situ), basal-cell carcinoma, or squamous-cell carcinoma. The later might arise in the hyperkeratotic areas or might appear on nonkeratotic areas of the trunk, extremities, or head (Sommers and McManus 1953; Yeh 1973).

A history of As exposure through inhalation or ingestion is helpful in corroborating a diagnosis of arsenicosis since skin manifestations such as diffuse melanosis can not be differentiated from normal dark complexioned farmers in the tropics who work in the field bare bodied under direct sunlight. However, spotty rain drop pigmentation of the skin distributed bilaterally and symmetrically over trunks and limbs is the best diagnostic feature of arsenical hyperpigmentation. Though spotty depigmented spots, similarly distributed are also diagnostic for this condition, sometimes blotchy depigmented spots are seen and these need to be differentiated from other depigmented skin lesions like tinea versicolor, seborrheic dermatitis. Diffuse hyperkeratitic lesions of the palms and soles are distinctive lesions of chronic arsenicosis. However, manual labourers, who work with bare hands, might have thickening of the palms. The thickening of palms in manual labourers are usually localised in the pressure points. Bare footed farmers who work in the fields might have diffuse thickening of the soles. However, when the lesions become nodular the diagnosis becomes obvious.

The duration of the patient's As exposure with the date of onset of symptoms does not follow a particular time frame. Arsenical skin lesions have been reported to occur in West Bengal after drinking As contaminated water for one year or even less (Garai et al, 1984, Guha Mazumder et al., 1997). In Taiwan, the youngest patient drinking As contaminated water who developed hyperpigmentation was 3 years old (USEPA 1988). Among the population exposed to As in drinking water in the Antofagasta region of Chile, cases of cutaneous arsenicosis, including both hyperpigmentation and hyperkeratosis, have been described in children as young as 2 years of age (Rosenberg 1974; Zaldivar and Guillier 1977). The mean As dose in Antofagasta was estimated to be approximately 0.06 mg/kg per day for subgroups of children aged 3.13 ± 3.33 years but was approximately 0.02 mg/kg per day for subgroups in their teens and twenties and 0.006 mg/kg per day for a subgroup in their sixties, indicating an inverse relationship between daily As dose rate/kg body weight and age (Zaldivar and Ghai 1980). In a retrospective study of 262 adults treated with Fowler's solution, Fierz (1965) reported the minimal latency period for hyperkeratosis to be 2.5 years, following ingestion of approximately 2.2 g of arsenite. Rattner and Dorne (1943) reported the development of hyperpigmentation within 6-12 months of the start of treatment with As at a dose of 4.75 mg/day. Hyperkeratosis appeared after approximately 3 years. Hence a history of chronic As exposure for more than 6 months is essential for diagnosis of As related skin manifestation.

With history of chronic As exposure and arsenical skin lesions, other indicators of chronic arsenicosis are weakness, anaemia, peripheral neuropathy, hepatomegaly with portal zone fibrosis (with/without portal hypertension), chronic lung disease and peripheral vascular disease (Espinosa 1963, Zaldivar 1974, Zaldivar & Ghai 1980, Datta 1976, Tseng 1977, Guha Mazumder et al. 1988, 1992, 1997, 1998a, Chen et al. 1988a, Engel and Smith 1994, Lagerkvist and Zitterlund 1994, Nins 1997, Kilburn 1997, Guo et al 1998). These features are manifested variably in different exposed populations and may also be caused by As unrelated conditions. Infrequent manifestations, which have been reported to occur by some investigators in people giving a history of chronic As exposure and which may be As unrelated are: conjunctivitis, keratitis, rhinitis, cardiovascular disease, gastrointestinal disease, hematological abnormalities, cerebrovascular disease, dysosmia, perceptive hearing loss, cataract, nephropathy, solid edema of the limbs, and diabetes mellitus (Tay and Seah 1975, Hotta 1989, Lai et al 1994, Gorby 1994, Morton and Dunnette 1994, Chen et al 1997, Guha Mazumder et al 1998a, Rahman et al 1998).

These have least diagnostic value of chronic As toxicity inspite of their reported occurrence amongst people with a history of chronic As exposure.

Proper investigations need to be carried out to define the various clinical manifestations of chronic arsenicosis. Routine investigations should include haematology (Hb, total and differential count, RBC morphology), urine and stool examination, chest X-ray, electrocardiogram, determination of blood sugar, urea and creatinine. Patients with hepatomegaly need further investigation such as tests for hepatitis B and hepatitis C, liver function, ultrasonography and liver biopsy. Those having history of chronic cough and/or dyspnoea should be investigated by lung function tests. People having features of restrictive lung disease need further investigation by high resolution CT scan for the diagnosis of interstitial lung disease or bronchiectasis. Testing of nerve conduction velocity and electromyogram would help in the diagnosis of peripheral neuropathy. Upper GI endoscopy need to be done in people presenting with features of dyspepsia and portal hypertension. Doppler study of peripheral vessels may help in the diagnosis of peripheral vascular disease.

That chronic arsenicosis produces protean manifestations is evident from the report of the clinical features in 156 cases who had been drinking As contaminated water in West Bengal (Guha Mazumder et al 1998a) (Table 4.1.1).

Table 4.1.1 : Clinical features of chronic toxicity; study of 156 cases in West Bengal.

Symptoms	No. of cases	(%)	Signs	No of cases	(%)
Weakness	110	(70.5)	Pigmentation	156	(100.0)
Headache	32	(20.5)	Keratosis	96	(61.5)
Burning of the eyes	69	(44.2)	Anaemia	74	(47.4)
Nausea	17	(10.9)	Hepatomegaly	120	(76.9)
Pain abdomen	60	(38.4)	Splenomegaly	49	(31.4)
* epigastric	39	(25.0)	Ascites	5	(3.0)
* paraumbilical	21	(13.4)	Pedal oedema	18	(11.5)
Diarrhoea	51	(32.6)	Sign of lung disease	45	(28.8)
Cough	89	(57.0)			
* with expectoration	53	(33.9)	Sign of polyneuropathy	21	(13.4)
* without expectoration	36	(23.1)			
Haemoptysis	8	(5.1)			
Dyspnoea	37	(23.7)			
Paresthesia	74	(47.4)			

Guha Mazumder et al. 1997.

Though pigmentation was seen in all cases, keratosis was found in 96 patients (61.5%), and skin cancer was detected in two (13%) cases. Weakness was a predominant symptom (70%) while anemia was present in 47% of cases. Nausea, anorexia, abdominal pain and diarrhoea were present in 91 patients (58.3%). Symptoms of respiratory disease were found in 89 (57.1%) cases. Lung function tests carried out on 17 patients showed features of restrictive lung disease in 9 and combined obstructive and restrictive lung disease in 7. Evidence of polyneuropathy was found in 79 (50.6%) cases. Objective evaluation of neuronal involvement could be done on 29 patients. Of these abnormal EMG was found in 10 (30.8%) and altered nerve conduction velocity and EMG in 11 (38%) cases. Perceptive hearing loss was found in two cases. Liver enlargement was found in 120 (76.9%) cases and was palpable 2-6 cm below the costal arch. Spleen was palpable 1.5-8 cm below the costal arch in 41 (31.4%) cases while ascites was present in 5 (3%) cases. Liver function tests could be done in 76 patients. Abnormal serum globulin (>3.5 gm/dl) level and alkaline phosphatase (>200 IU/dl) values were found in 12 (15.8%) and 39 (51.3%) cases respectively. Significant elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were found in 9 (11.8%) and 21 (27.6%) cases respectively. Biopsy reports were available from 45 patients. Non-cirrhotic portal fibrosis was found on histology in 41 cases and cirrhosis in 2 cases while normal histology was observed in 2 patients. The liver histology of noncirrhotic portal fibrosis (NCPF) was characterized by expansion of the portal zone of varying degrees (Figure 4.1.5 - Liver histology of a case of chronic arsenicosis showing fibrous expansion of portal zone with extension in the liver lobule (H & E). (Guha Mazumder DN & Ghosh AK, personal collection) (PENDING)). Fine to thick stellate scars were found to spread out of the portal tracts which frequently contained leash of vessels. There was paucity of inflammatory cells in the portal zone and absence of gross hepatocellular damage. The fibrosis in the liver was mostly found to be mild (Grade-I 53.6%, Grade-II 29.6%) while moderate to severe fibrosis was found in a smaller number of cases (Grade-III 9.75% and Grade – IV 7.31%) (Fig. 4.1.6 - Various grades of noncirrhotic portal fibrosis of liver in chronic arsenicosis. Grade I and II (upper panel) Grade III and IV (lower panel) (Reticulin Stain) (Guha Mazumder DN & Ghosh AK, personal collection) (PENDING)). Portal hypertension was found in 52 cases (33.3%) as evidenced by splenomegaly and/or esophageal varices. However only three of these patients had hematemesis and melena. Except for lowered blood hemoglobin, no other hematological abnormality was detected in any of the cases. Urine reports and blood sugar, urea and creatinine values were found to be within normal limits. Peripheral vascular disease was detected in 3 cases when 64 more patients from severely affected area have been further investigated.

As exposure is a major risk factor for blackfoot disease, a unique peripheral arterial disease characterized by the severe systemic arteriosclerosis as well as dry gangrene and spontaneous amputations of affected extremities at end stages (Tseng, 1977; Chen et al., 1988a). Diagnostic criteria for blackfoot disease include objective signs of ischemia, i.e., absence or diminution of arterial pulsation, pallor on elevation or rubor on dependency of ischemic extremities, and various degrees of ischemic changes in the skin, as well as subjective symptoms of ischemia, i.e., intermittent claudication, pain at rest, and ischemic neuropathy. Not all patients are affected with black, mummified dry gangrene (Tseng et al., 1961). Extensive pathological study showed that 30% of blackfoot disease patients had histological lesions compatible with thromboangiitis obliterans, and 70% showed changes of arteriosclerosis obliterans. Marked generalized atherosclerosis was observed in all autopsied cases of blackfoot disease. Any of the fundamental vascular changes of the disease represent an unduly developed severe arteriosclerosis (Yeh and How, 1963). A recent study has shown a dose-response relationship between cumulative As exposure and subclinical peripheral vascular disorder detected by Doppler ultrasonography among seemingly normal subjects after cessation of drinking artesian well water in the endemic area of blackfoot disease in Taiwan (Tseng et al., 1995a).

Skin cancer of chronic arsenicosis is quite distinctive. The lesions are frequently multiple and involve covered areas of the body, contrary to non arsenical skin cancer which usually presents as a single lesion and which occur in exposed parts of the body (Tseng, 1977; Zaldivar et al 1981). Though other types of cancers, e.g. lung cancer, bladder cancer, kidney cancer, prostate cancer, angiosarcoma of the liver are observed in significantly higher number among cases of chronic arsenicosis (NRC 1999), these have no characteristic feature suggestive of arsenic etiology.

The As content of water consumed by patients with involvement of major organ system, as studied by Guha Mazumder et al 1997 is shown in Fig. 4.1.7 (Various levels of arsenic in drinking water and its relation with initial presentation. (Guha Mazumder, et al, 1997).(PENDING) Most of the patients had keratosis and hepatomegaly when As concentrations levels in drinking water were more than 0.5 mg/L. On the other hand a number of people did not have any lung or neurological manifestation even when they were drinking water containing more than 1 mg/L As. Thus keratosis and hepatomegaly have more diagnostic specificity than neurological or respiratory manifestations of chronic As toxicity. Since hepatomegaly may be caused by many other factors, it is not a specific indicator of As exposure. Because few

conditions cause keratotic lesions in the skin these are most diagnostic for chronic arsenicosis. Other biomarkers for chronic As toxicity such as micronuclei, sister chromatid exchange and hprt mutant frequency have been described but are not specific for As. Given the relationship of skin cancer and hyperkeratosis observed in Taiwan, a dose-response analysis of hyperkeratosis in a US population exposed to As was found to be consistent with the EPA (US) skin cancer dose response estimate made from the Taiwan data (Chen and Chen 1991). Hyperkeratotic lesions occur much earlier following As exposure than does skin cancer and are much more prevalent. In the Tseng study (Tseng et al. 1968) of an As endemic area in Taiwan, the youngest person with hyperkeratosis was 4 year old; the youngest skin cancer case was 23 years of age. Hyperkeratosis was almost 20 times more prevalent in the As exposed population than skin cancer. Further, according to some, skin cancer arises from hyperkeratotic lesion (Yeh, 1973). This hyperkeratosis occurs more commonly and earlier in an As exposed population than does skin cancer. A dose response analysis of hyperkeratotic lesions may therefore allow one to observe potential carcinogenic response at lower exposures than has been done with skin cancer. Necessary information for the risk assessor to estimate dose-response would be the length and intensity of exposure and the prevalence (or incidence if possible) of hyperkeratosis by exposure and age (H. Gibbs in North et al 1997).

It becomes evident that with the exception of cutaneous manifestations other symptoms and signs of chronic arsenicosis are non specific and can occur with other unrelated medical conditions. Hence, history of As exposure by drinking As contaminated water and high level of As in urine and/or in hair and nails in association with those symptoms may help in the diagnosis of chronic arsenicosis. But its normal value in those materials do not exclude such diagnosis. Diagnostic criteria, grading of severity of dermatological manifestations and case definition of chronic As toxicity are summarised in the Tables 4.1.2, 4.1.3 and 4.1.4.

Table 4.1.2. Diagnostic criteria of Chronic arsenicosis.

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1. At least 6 months exposure to arsenic levels of greater than 50 µg/L or exposure of high arsenic level from food and air.
 2. Dermatological features characteristic of chronic arsenicosis.
 3. Non carcinomatous manifestations : Weakness, chronic lung disease, non cirrhotic portal fibrosis of liver with/without portal hypertension, peripheral neuropathy, peripheral vascular disease, non pitting edema of feet/ hand.
 4. Cancers : Bowens disease, Squamous cell carcinoma, Basal cell carcinoma at multiple sites, occurring in unexposed parts of the body.
 5. Arsenic level in hair and nail above 1 mg/kg and 1.08 mg/kg respectively and/or arsenic level in urine, above 50 µg/L (without any history of taking seafood).
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Table 4.1.3. Dermatological criteria and grading of severity of chronic arsenic toxicity.

Grade I	Mild	a) Diffuse melanosis. b) Suspicious spotty depigmentation / pigmentation over trunk /limbs. c) Mild diffuse thickening of soles and palms.
Grade II	Moderate	a) Definite spotty pigmentation / depigmentation on the trunk and limbs, bilaterally distributed. b) Severe diffuse thickening (with/without wart like nodules of the palms and soles).
Grade III.	Severe	a) Definite spotty pigmentation/depigmentation as above with few blotchy pigmented/depigmented macular patches over trunks or limbs. b) Pigmentation involving the undersurface of tongue and/or buccal mucosa. c) Larger nodules over thickened palms and soles occasionally over dorsal aspect of hands and feet. Diffuse verrucous lesions of the soles with cracks and fissures and keratotic horns over palms/soles.

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Table 4.1.4. Case definition of chronic arsenic toxicity.

Definite

1. Criteria 1 + Criteria 2 ± Criteria 3 ± Criteria 4 + Criteria 5
 2. Criteria 1 + Criteria 2 (Grade II/ III) ± Criteria 3 ± Criteria 4
 3. Criteria 2 (Grade II / III) ± Criteria 3 ± Criteria 4 + Criteria 5
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Probable.

1. Criteria 1 + Criteria 2 (Grade I) ± Criteria 3 ± Criteria 4
 2. Criteria 2 (Grade I) ± Criteria 3 ± Criteria 4 + Criteria 5
 3. Criteria 2 (Grade II/III) ± Criteria 3 ± Criteria 4
 4. Criteria 3 + Criteria 5
 5. Criteria 4+ Criteria 5
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Guha Mazumder et al. (In press)

A few epidemiological studies have highlighted that none of the exposed population to environmental As show any clinical manifestation of chronic As toxicity. (Goldsmith 1980. Harrington et al 1978. Valentine et al 1985). Further, there is a wide variation in the incidence of chronic arsenicosis in an affected population. Even not all members of an affected family show clinical effect. The reasons for such variation of disease expression is an enigma. However, as the As exposed people are at risk for developing As related cancer, they should be subjected to prolonged surveillance.

4.2. Biomarkers with special focus exclusively on diagnosis.

On the basis of As metabolism data, important biomarkers of internal exposure are: the urinary excretion of the element and its concentration in hair and nail (blood concentrations are generally too low and transient). Despite some encouraging reports, the use of As measurements in hair and nail as indices of absorbed dose appears limited. Efforts are needed to develop a standardized procedure to solve the problem of external contamination of samples. The relationship between As air concentration and urinary excretion of inorganic arsenic, and of mono and dimethyl arsonic acid, appears better. As urinary excretion (seafood As excluded) as a function of As oral intake via drinking water in steady state conditions, has been reported by several authors from different countries. Despite possible ethnic and environmental differences,

reported results display a quite satisfactory consistency. Most strikingly, an increased excretion rate is observed when the water As concentration reaches 100 - 200 µg/L (Buchet and Hoet, 1998).

4.2.1. Urine

The concentration of total As in urine has often been used as an indicator of recent As exposure because urine is the main route of excretion of most As species (Buchet et al. 1981, Vahter 1994). The half-time of inorganic As in humans is about 4 days. Average background concentrations of As in urine are generally below 10 µg/L in Europe (Apel and Stoepler 1983; Valkonen et al. 1983; Foa et al. 1984; Vahter et al 1986; Andren et al. 1988; Jensen et al. 1991; Buchet et al. 1996; Trepka et al. 1996; Kristiansen et al. 1997; Kavanagh et al. 1998), somewhat higher in some parts of the US in people living near point source emissions, especially copper smelters (Smith et al. 1977; Morse et al. 1979; Binder et al. 1987), and around 50 µg/L in Japan (Yamauchi and Yamamura 1979; Yamauchi et al. 1992). In certain areas in the US, an average concentration of As of 10 µg/L or less has been reported for children (Kalman et al. 1990; Pollisar et al. 1990; Gottlieb et al. 1993).

Urinary As concentrations have also been shown to correlate with As intake in drinking-water. A survey was conducted by Harrington, et al. (1978) among a population in an area with elevated As concentrations in well water. Drinking well water with an As content exceeding 100 µg/L (mean 401 µg/L and an estimated total daily intake of 324 µg/L of As) gave an average urinary total As concentration of 178 µg/L (atomic absorption spectrophotometry). Drinkers of well water containing an average As concentration of 31 µg/L (estimated daily intake of 46 µg of As) had a mean urinary As concentration of 41 µg/L.

Seafood in the diet may influence urinary As measurements. Certain seafoods (particularly cold water fin fish, crustaceans, and molluscs) may contain large amounts of organo arsenic compounds, that have no known mammalian toxicity. In addition, certain edible marine foods, such as seaweed or kelp, may contain arsenosugars that are without recognized toxicity. These compounds are well absorbed from the gastrointestinal tract, and in the case of arsenobetaine, are largely excreted unchanged in the urine. When a clinical laboratory measures and reports the total As content in urine, the value may be markedly elevated (up to hundreds or thousands of µg/L) if

they have ingested seafood within the past 1-2 days. In an effort to avoid the contribution of complex organo arsenicals in seafood, some clinical laboratories use a speciation method that only measures inorganic As, or its metabolites, monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA). However, certain marine organisms, particularly bivalves such as clams, may contain over one hundred micrograms of dimethylarsinic acid in a typical serving, and may thus elevate urine As values even when the more restrictive speciation methods of analysis are used. Consequently, a urine As measurement may not be a valid reflection of As ingestion from drinking water if there has been any consumption of seafood (including seaweed products) within the past three days.

The pattern of As species in urine from individuals chronically exposed to high concentrations of As via drinking water in Region Lagunera, Mexico was studied by Del Razo et al (1994). The urinary concentrations of As species and consequently of total As, were significantly higher in the exposed group. The sum of As species accounted for at least 95% of total As in urine. No significant differences in the proportion of inorganic and total organic As compounds in urine were observed (Table 4.2.1.1).

Table 4.2.1.1 : Arsenic species excreted in urine from humans chronically exposed to arsenic (geometric mean (range)).

		CONTROL	EXPOSED
Total As	($\mu\text{g g}^{-1}$ creat.)	39.3 (12-104)	1,156 ¹ (456-1,981)
Inorganic As	($\mu\text{g g}^{-1}$ creat.)	5.6 (0.5-22)	187.9 ¹ (34-503)
	%	10.9 (1.4-29.5)	16.7 (9.3-25.5)
Organic As	($\mu\text{g g}^{-1}$ creat.)	35.1 (8.4-103.4)	834.8 ¹ (234.2-1,832)
	%	84.8 (70.4-98.6)	82 (74.5-96.8)
MMA	($\mu\text{g g}^{-1}$ creat.)	3.24 (0.7-16.6)	189.4 ¹ (44-645)
	%	7.12 (1.8-2.5)	18.42 ¹ (6.2-29.5)
DMA	($\mu\text{g g}^{-1}$ creat.)	30.56 (5.7-93.4)	680.21 ² (187-1,264)
	%	74.8 (46.8-94.6)	61.73 ² (47.9-81.8)
n		22	23

Del Razo LM et al. 1997.

¹ p = 0.00002. Mann – Whitney’s U test

² p = 0.002. Mann-Whitney’s U test.

However, detailed study of the organic species showed that the exposed group had a significant increase in the proportion of MMA excreted in urine, accompanied by a significant decrease in the proportion of DMA excreted (Figure 4.2.1.1.- Proportions of arsenic species in urine of individuals chronically exposed to arsenic via drinking water. Region Lagunera, Mexico. (Del Razo, et al, 1994).(PENDING). Nonetheless, DMA was the major single As species excreted by both groups. The mean percentages of urinary inorganic As were within the ranges described for two other populations chronically exposed to relatively large amounts of As in drinking water,

one in Nevada, USA (Warner et al 1994) and the other in San Pedro, Chile (Hopenhayn-Rich et al., 1996).

Because of the variations in the proportions of different As metabolites in urine, sum of the metabolites is a better indicator of exposure than the concentration of inorganic As or DMA in urine. The exact reasons for the variations are largely unknown, but probable influences are age, sex, health status, genetic factors, and analytical variability. Genetic polymorphism of the still uncharacterized As methylation enzymes may help explain the inter individual variation. Similar genetic differences may exist in arsenic-specific binding proteins, which are thought to decrease the toxicity of inorganic As by decreasing its tissue availability until it can be methylated (Bogdan GM et al 1994). In a population, group-average concentrations of As metabolites in the urine correlate with the average concentrations of As in drinking water. However, the relationship can vary considerably depending on the amount of water consumed and the amount of water used for cooking.

For measuring concentrations of exposure markers in the urine, an important question is whether to collect 24-hr urine samples, spot urine samples, or early morning urine samples. Ideally, the amount of As excreted over a certain period of time should be assessed. Usually this is done by measuring As excretion in a 24 hr collection. However, obtaining complete 24-hr urine collections may be difficult (Bingham and Cummings 1983; Johansson et al. 1998) and requires supervision and validation. Because of these difficulties and other problems (e.g. the risk of contamination of the urine during sampling), the first-morning urine or spot urine samples are generally collected for measurement of the urinary concentration of inorganic As or As metabolites. There are several reasons why a single spot urine measurement, despite, its limitations could reflect the 'usual' dose in the study population. Under chronic exposure conditions, one can assume that the participants were exposed to As in a fairly constant manner. To evaluate the concentration properly, especially in the case of spot urine samples, the dilution of the urine has to be considered. The urine flow is highly variable, being dependent on numerous factors, such as body size, body water content, solute intake, physical activity, and diurnal variation (Diamond 1988). A short time after the consumption of large amounts of fluid, the urine is very diluted and has a low solute content. To compensate for the dilution, the concentration of As species can be related to the concentration of creatinine or the specific gravity. A disadvantage of using the creatinine-adjusted urinary As measurement is that it is dependent on the muscle

mass and thus is often quite different for men and women. Protein intake might also influence urinary creatinine concentration.

Though high AS excretion in urine is indicative of continued As exposure, this is not always diagnostic of chronic As toxicity. In West Bengal, India, a significant number of people who were drinking As contaminated water and had high urinary As excretion did not show cutaneous manifestations of chronic As toxicity (9 out of 17). On the other hand many of the chronically As exposed people showing arsenical skin lesions did not have high urinary As excretion (33 out of 40). (Chowdhury TR et al. 1997). These results might be explained by the fact that all those who are drinking As contaminated water at a point of time may not be showing clinical features of chronic As toxicity, while others who might have taken As contaminated water for a long time in the past but have then stopped drinking contaminated water might still have clinical expression of As toxicity.

4.2.2. Hair and Nail.

Arsenic is normally found in higher concentrations in human hair and nails than in other parts of the body. This has been explained by the high content of keratin in these tissues (Shapiro, 1967). Hair As levels can provide useful information in chronic As poisoning but undue weight should not be given to the results. Several problems confront the toxicologist when using this test: there is only a very approximate relationship between hair As concentration and As toxicity. Thus, patients with chronic As poisoning may have hair concentrations varying, from 10 ppm (10 mg/kg hair) to 100 ppm whereas levels of around 45 ppm have been reported in Aa-related fatalities. Results derived from the analysis of a single hair or of one site along the shaft of a single hair are much less reliable than mean levels from larger hair samples because the inter and intra-hair variations in As content can be very large. Thus, samples should consist of at least one gram of hair cut close to the scalp and derived from several sites on the head and the whole sample should be analysed. External contamination of the hair by As must be excluded in order to use hair As concentrations to assess toxicity. Ingested As and As derived from external contamination are both avidly bound to the outer surface of the hair and these two sources cannot be differentiated by any known technique. External contamination can produce As concentrations of several thousand ppm and therefore can mislead investigators attempting to diagnose chronic As poisoning. Despite these pitfalls, the test can give useful information if carefully interpreted (Hindmash JT, 1998).

In people with no known exposure to As the concentration of As in hair is generally 0.02-0.2 mg/kg (Valentine et al. 1979; Olguin et al. 1983; Narang et al. 1987; Takagi et al. 1988; Koons and Peters 1994; Wang et al. 1994; Wolfspurger et al. 1994; Vienna et al. 1995; Raie 1996; Paulsen et al. 1996; Rogers et al. 1997; Kurtio et al. 1998). The concentrations of As in hair are clearly increased in people drinking water with high As concentration. For example, concentrations ranging from 3 to 10 mg/kg are reportedly common in people in areas in West Bengal that have high As concentrations in drinking water (Das et al. 1995).

On a group basis, a few reports indicate that the correlation between the concentration of As in drinking water and the concentration in hair is fairly good, although it is not known how much of the As in hair originates from As in blood and how much is bound due to external contact with the water, as discussed above. In studies carried out in California and Nevada, a concentration of 400 µg/L in drinking water corresponded to about 1.2 mg/kg in hair and 100 µg/L in water corresponded to about 0.5 mg/kg in hair (Valentine et al. 1979). In Alaska, an average of 400 µg/L in drinking water corresponded to 3.3 mg/kg in hair (Harrington et al. 1978). In Hungary, people with drinking-water concentrations ranging from 50 to 100 µg/L had an average hair concentration of 3 mg/kg (Borzsonyi et al. 1992). The highest hair As concentrations were found in children (Grantham and Jones 1977).

Normal As values in nails appear to range from 0.02 to 0.5 mg/kg (Narang et al. 1987; Takagi et al. 1988). Several tens of mg per kg have been reported in cases of chronic poisoning (Pounds et al. 1979; Das et al. 1995). A single dose of As can be detected at the distal tip of the nails about 100 days after exposure (Pounds et al. 1979; Pirl et al. 1983). Presumably, As is deposited in the nail roots from the blood and then migrates distally as the nails grow (at about 0.12 mm a day).

In one case of repeated ingestion of As over a period of one year, the value of sectional nail analysis was investigated by Henke et al. (1982). The As determinations were performed by instrumental neutron activation analysis. After subdividing the nail transversely into segments of 0.5 mm length, several maxima and minima of As concentrations were found. Taking the nail growth into consideration, the results corresponded to the known dates of treatment and discharge from hospital. The results excluded external contamination of the nail.

Although hair/nail As has been found to be elevated in people drinking As contaminated water there is no correlation between As concentration in hair and nail and the degree of exposure

(Guha Mazumder et al. 1997). Similarly there is no correlation between hair and nails As and clinical features of chronic As toxicity. In a village of West Bengal all 17 people drinking As contaminated water had raised hair and nail As, but only 8 had cutaneous lesions. Further, out of 40 people with arsenical skin lesions in another village of West Bengal with a history of drinking As contaminated water, normal hair and nail As was found in 31 and 26 cases respectively (Chowdhury et al. 1997).

4.2.3. Blood

Most inorganic and organic As in blood is cleared fairly rapidly in man. Blood As will therefore reflect exposure for only a short period following absorption and will be very time dependent. Only if exposure is continuous and steady, as is sometimes the case with exposure through drinking-water, will As reach steady-state in the blood and thus make it possible to discern a relationship between blood As and exposure. Even so, there are no data that indicate a quantitative relationship in man between As exposure and blood As concentrations. The short half-life of As in the blood compared with the half-life in the body makes it difficult to discern a relationship between blood As concentration and total body As burden or As concentrations in different organs.

Partial speciation of As in blood has been reported in few cases (Zhang et al. 1996; Concha et al. 1998b). When using total As in blood as an indicator of exposure to inorganic As, the interference from organic As compounds originating from seafood has to be considered. Furthermore, because of the low concentrations, the analytical error might be significant, unless relatively sensitive methods are used. Data on concentrations of As in blood in people with no known exposure to As are in the range 0.3 – 2 µg/L (Bencko and Symon 1977; Heydorn 1970; Kagey et al. 1977; Olguin et al. 1983; Hamilton et al. 1994; Vahter et al. 1995; Concha et al. 1998a,b).

In people exposed to As in drinking water (200 µg/L) in northern Argentina, the mean blood As concentration was about 10 µg/L (Vahter et al. 1995; Concha et al. 1998a,b). In studies carried out in California and Nevada, an As concentration of 400 µg/L in water corresponded to about 13 µg/L in blood, and 100 µg/L in water corresponded to 3-4 µg/L in blood (Valentine et al. 1979). Obviously, compared with urine, blood is a much less sensitive biomarker of exposure to As via drinking water (NRC 1999).

4.2.4. Other Tissues.

The concentrations of As in various human tissues determined by neutron activation analysis and reported by Liebscher & Smith (1968), Larsen et al. (1972), and Brune et al. (1980) are shown in Table 4.2.4.1.

Table 4.2.4.1. : Arsenic concentration in human organs and tissues.

Tissue or organ	Dry weight ^a (geometric mean values)	Arsenic concentration (mg/kg)	
		Wet weight ^b (mean values)	Wet weight ^c (median values)
adrenal	0.03		
aorta	0.04		
whole blood	0.04		
brain	0.01		
hair	0.46		
heart	0.02		
kidney	0.03	0.007	0.004
liver	0.03	0.011	0.003
lung	0.08	0.010	0.008
muscle	0.06 (pectoral)	0.004	
nail	0.28		
ovary	0.05		
pancreas	0.05	0.005	
prostate	0.04		
skin	0.08		
spleen	0.02	0.003	
stomach	0.02		
teeth	0.05		
thymus	0.02		
thyroid	0.04		
uterus	0.04		

WHO, 1981.

a Compiled from Liebscher & Smith (1968).

b Compiled from Larsen et al. (1972).

c Compiled from Brune et al. (1980).

Several autopsy studies have linked exposure to inhaled As in smelter workers with persistence of As in lung (Brune et al 1980, Gerhardsson et al, 1988). In one study, exposed workers had lung As concentrations six times higher than in controls (47 $\mu\text{g}/\text{kg}$ tissue versus 8 $\mu\text{g}/\text{kg}$). These increases were not seen consistently in the kidney or in the liver, and the elevation in the lung did not decline significantly even as the time from retirement to death increased, suggesting a long half-life (Brune et al, 1980). Other evidence also indicates that ingested As reaches the lungs. A fatal poisoning following As ingestion by a 3 year old boy resulted in an As concentration in the lungs of 7550 $\mu\text{g}/\text{kg}$ (Saddy et al 1989). In another fatal case the As lung concentration was 2750 $\mu\text{g}/\text{kg}$ (Quatrehomme et al. 1992).

Maximum As content of the liver in people with hepatomegaly drinking As contaminated water in West Bengal, India, was 6 mg/kg (neutron activation analysis) although As was undetectable in 6 out of 21 case samples tested (mean 1.39 ± 0.3 mg/kg, control 0.016 ± 0.04 mg/kg). There was no correlation between the As content in biological tissues (liver, hair and nails) and the As dose taken by the patients (Fig. 4.2.4.1 - Correlation of arsenic content in biological tissue (Liver, Hair & Nail) with quantum of arsenic exposure. (Guha Mazumder et al, 1997) (PENDING)).

4.3. Treatment of chronic Arsenic toxicity.

Chronic arsenicosis leads to irreversible damage in several vital organs and As is an established carcinogen. Despite the magnitude of this potentially fatal toxicity, there is no effective therapy for this disease; patients once affected may not recover even after remediation of the As contaminated water. The need for an effective therapy for chronic arsenicosis is obvious.

Chelation therapy for chronic As toxicity is thought to be the specific therapy for relief of systemic clinical manifestations and reduction of As stores in the body, reducing subsequent cancer risk. Chelation therapy is presumed to be more effective with early features of the toxicity, as severe manifestation of polyneuropathy, chronic lung and liver disease, swelling of hand and legs, defect of hearing and vision are less likely to respond to this therapy. Chelating agents like, DMSA (Dimercaptosuccinic Acid), DMPS (Dimercaptopropane succinate) d-penicillamine have frequently been considered for treatment of chronic As toxicity. However, their usefulness are yet to be established.

4.3.1. Chelators.

A chelating agent forms ring structure with a metal or metalloid. When used for treating heavy metal poisoning, the administration of the chelating agent results in the formation of a chelate structure which has a water solubility greater than that of the offending metal and thus increases its excretion by the kidney. The chelating agent usually has a greater affinity for the metal ion than do endogenous ligands to which the offending metal is bound. A number of chelating agents are considered for use against As poisoning.

4.3.1.1. DMSA (meso-2,3-dimercaptosuccinic acid, Succimer, Chemet) and DMPS (sodium 2,3 – dimercapto-1-propane sulfonic acid, Dimaval) (Fig. 4.3.1.1.1 - Chemical formula for chelating agents used for treating heavy metal poisoning in humans - PENDING)

DMSA and DMPS are water soluble analogues of dimercaprol developed as heavy metal chelators in the 1950s (Liang et al 1957, Petrunkin 1956). Evaluations of poisoning by lewisite in rabbits (Inns et al 1990, Inns and Rice 1993) and As trioxide in mice (Kreppel et al 1990, 1993) and in guinea pigs (Reichle et al 1991) showed better results by treatment with DMSA and DMPS over BAL (British anti Lewisite). Reichl et al (1992) reported that the biliary excretion of As from perfused guinea pig liver increased from 0.1% with BAL to 12.3% with DMPS. The significantly lower toxicity, the ease of oral administration, and the enhanced biliary clearance of As, all contribute to the clinical consensus that DMSA and DMPS and not BAL are the first choice for As poisoning (Kelafant et al 1993, Kew et al 1993, Marcovigi et al 1993). DMPS appears to be biotransformed in humans to acyclic and cyclic disulfides. Whereas DMSA in humans is biotransformed almost completely to a DMSA: CySH (1:2) mixed disulfide (Maiorino et al 1989), a DMPS-Cysteine mixed disulfide has been found only in minute amounts after administration of DMPS. Another difference between DMSA and DMPS is that the later is distributed both in an extracellular and to a small extent an intracellular manner while the former is distributed only extracellularly (Zheng et al 1990, Wildenauer et al 1982, Reuther et al, 1982). Both renal and biliary excretion of DMPS occur (Zheng et al, 1990).

Controlled animal experiments have demonstrated that dimercaprol, DMSA, and DMPS increase survival when administered within minutes to hours after acute poisoning with lethal doses of organic or inorganic arsenicals (Stocken and Thompson, 1946; Tadlock and Aposhian, 1980). However, the efficacy of these agents declined in proportion to the length of time after acute As

exposure before that treatment was begun. In studies of the effect of dimercaprol on experimental organoarsenical poisoning in rabbits, Eagle et al (1946) noted that all animals survived when a single injection of dimercaprol was administered 5 minutes after the exposure of arsenical, compared to no survival if treatment was delayed for 6 hours. Data obtained by Tadlock and Aposhian (1980) on the efficacy of single dose of DMSA (0.25 mmol/kg, i.p.) against a lethal dose of sodium arsenite (0.14 mg/kg, s.c.) in mice suggests that beneficial effects on survival may begin to diminish when treatment is delayed for 2 or more hours.

The first prospective randomised controlled trial to evaluate the efficacy and safety of dimercaptosuccinic acid (DMSA) to chronic arsenicosis patients was carried out by Guha Mazumder et al. (1998c). Twenty-one consecutive patients with chronic arsenicosis were randomized into 2 groups. Eleven patients (10 males, ages 25.5 ± 8.0 years) received DMSA 1400 mg/d (1000 mg/m^2) in 4 divided doses in the first week and then 1050 mg/d (750 mg/m^2) in 3 divided doses during the next 2 weeks. The same was repeated after 3 weeks during which no drug was administered. The other 10 patients (all males, ages 32.2 ± 9.7 years) were given placebo capsules (resembling DMSA) in the same schedule. The patients were blinded about the nature of treatment being given. The patients included in the study were selected from the As clinic on the basis of history of drinking As contaminated water ($\geq 0.05 \text{ mg/L}$) for 2 years or more and clinical symptoms and signs of chronic arsenicosis. The symptoms and signs of patients were evaluated by a scoring system before and after treatment (c.f. Table 4.3.1.1.1)

Table 4.3.1.1.1. : System of Clinical Scoring of the Symptoms and Signs Before and After Therapy with DMSA and Placebo.

Symptoms and Signs	None	Mild	Present Moderate	Severe
Weakness	0	1		
Cough	0	1		
Dyspnea	0	1	2	3
Rales, rhonchi	0	1		
Hepatomegaly	0	1 (14 cm span)	2 (16 cm)	3 (> 16 cm)
Splenomegaly	0	1 (2 cm)	2 (4 cm)	3 (> 4cm)
Pigmentation	0	1 (Diffuse)	2 (Spotty)	3 (Blotchy)
Keratosi	0	1 (Thickening)	2 (Few nodules)	3 (Multiple nodules)
Flushing of face	0	1		
Conjunctiviti	0	1		
Edema leg/hand	0	1		
Abdominal pain	0	1		
Anorexia	0	1		
Nausea	0	1		
Diarrhea	0	1		
Hearing defect	0	1		
Claudication	0	1		
Hand/leg ulcers	0	1		
Paresthesia	0	1 (Only legs)	2 (Leg + hands)	
Pallor	0	1		
Ascites	0	1		
Loss of ankle jerk	0	1		

Guha Mazumder et al., 1998c

Maximum score 33.

Any possible therapy-related side effect was monitored in every patient. All the patients were kept hospitalized during the study period. Skin was biopsied from unexposed areas by punch biopsy technique for histologic evaluation before and after treatment. Urine samples were collected for 2 consecutive days before, and then at 48 and 72 hours after starting the drug or placebo. Urine As was determined by graphite furnace atomic absorption with Zeeman-background correction.

There were no differences in age, sex, duration of exposure to the As contaminated water, As concentration in the drinking water, duration of drinking As free water before inclusion into the study, and clinical score of symptoms and signs between patients on the drug and in controls. Therapy with DMSA did not cause any significant clinical improvement as compared to patients

treated with placebo. The clinical score improved after therapy with DMSA, but similar improvement was observed in patients treated with placebo (Table 4.3.1.1.2).

Table 4.3.1.1.2 : Clinical scores of patients before and after therapy.

	Before	After	p value
DMSA n = 11	9.33±3.33	6.2±2.11	0.017
Control n=10	10.6±3.20	6.7±1.70	0.003

(Guha Mazumder et al. 1988)

(Fig. - 4.3.1.1.2 - Clinical score of DMSA and placebo treated cases before and after therapy. (Guha Mazumder et al. 1998c) (PENDING)) There was no difference in the results of the urinary excretion of As, liver function tests and As concentration in hair and nails before and after treatment. No patient developed any therapy related side effects. The histologic abnormalities in skin biopsy did not show any difference in patients treated with DMSA and placebo before and after therapy. In this study the authors did not find DMSA for 2 courses at 3 week intervals to have any clinical or biochemical benefit in patients with chronic arsenicosis.

Shum and Whitehead (Shum S et al. 1995) reported that treatment of an adult who had ingested 80 g methane arsenate with DMSA 30 mg.kg/d x 5d over 1 month reduced serum As from 2871 µg/L to 6 µg/L. Lenz et al (Lenz K et al, 1981) also found DMSA to be effective in man. However, Kew et al (1993) found no improvement in peripheral neuropathy of 4 months duration after DMPS 300 g/d x 3 weeks and DMSA 1.2 g/d x 2 weeks.

In a recent study (Aposhian et al. 1997), the administration of DMPS to subjects with very recent, long-term ingestion of As in drinking water was found to be associated with a prompt increase in the excretion of As in the urine that was several fold above pre-chelation levels. In 13 subjects consuming As in drinking water (528 µg/L) until one day prior to the administration of a single oral 300 mg dose of DMPS, total urine As increased from a baseline of 605±81 µg/g creatinine

(Cr) to a peak of 2325 ± 258 $\mu\text{g/g}$ Cr in the first two hours post chelator. In 11 control subjects chronically consuming water containing As at a concentration of 21 $\mu\text{g/L}$, DMPS resulted in the baseline urine As concentration of 91 ± 17 $\mu\text{g/g}$ Cr transiently increasing to 305 ± 79 $\mu\text{g/g}$ Cr. The data are consistent with chelation accelerating the decorporation of As in chronically exposed humans. However, animal experiments suggest that compared to cessation of exposure alone, DMPS chelation may predominantly effect the rate of As excretion, rather than longterm net excretion (Maiorino and Aposhian, 1985).

Recently Guha Mazumder et al. (1998d) presented their preliminary data on the efficacy of treatment of DMPS in a single blind placebo controlled trial in patients suffering from chronic As toxicity in West Bengal. The trial design was similar to that carried out in DMSA trial (vide supra). DMPS was given in a dose of 100 mg capsules 4 time a day for a course of 7 days for four courses with one week drug free period between each course. Nine patients received the drugs, while 6 patients received placebo capsules. Baseline data and clinical scores before and after the treatment are given in table 4.3.1.1.3.

Table 4.3.1.1.3. Clinical score of patients pre and post therapy with DMPS and placebo.

	DMPS Treated group (n = 9)	Placebo group (n = 6)	P value
Age (year)	31.11 ± 12.18	31.00 ± 7.24	
Sex (M:F)	7:2	4:2	
As level in drinking water mg/l	0.60 ± 0.40	1.38 ± 1.07	ns
Duration (in years)	20.1 ± 11.24	18.3 ± 3.35	ns
Clinical score :			
Pre treatment	14.8 ± 8	13.2 ± 3.1	ns
Post treatment	4.3 ± 1.8^a	9.6 ± 0.96^b	$p < 0.01$

(Guha Mazumder et al.1998d.

- a $p < 0.01$ pre and post treatment DMPS
b $p < 0.05$ pre and post treatment placebo.

Though there was significant decrease of clinical score from pretreatment to post treatment values amongst both DMPS and placebo groups, there was significant difference in decrement of clinical score among DMPS treatment patients compared to placebo group (Fig. 4.3.1.1.3 - Clinical score of patients pre- and post therapy with DMPS and placebo. (Guha Mazumder et al, unpublished data) (PENDING)). However, there was no change of skin histology score of pre and post treatment skin biopsy carried out on 4 DMPS and 3 placebo treated cases. There was also no significant difference in the hematological and liver function test parameters amongst both the groups of patients before and after therapy with either DMPS or placebo. No side effects were noticed among the patients treated with DMPS. From the preliminary analysis of the data it appears that DMPS is more effective than placebo in improving clinical features of chronic As toxicity. However, follow up study of the cases treated need to be carried out to assess the efficacy of this initial improvement of clinical symptoms in altering the natural history of chronic As toxicity. At the present time, there is no follow-up data available to determine whether a short term increase in urinary As excretion associated with chelation will result in a lower risk of long term adverse outcomes, such as cancer (Kosnett MJ in press).

4.3.1.2. D-Penicillamine.

Penicillamine was first isolated in 1953 from the urine of patients with liver disease who were receiving penicillin. It is an effective chelator of copper, mercury, zinc and lead and promotes the excretion of these metals in the urine. The usual dose is 1 to 1.5 gm per day. The drug has been suggested for the treatment of long term exposure to arsenic either alone or in combination with dimercaprol. However with long term use, penicillamine induces several cutaneous lesions including urticaria, macular or papular lesion, pemphigoid lesion, lupus erythematosus. Hematological system also may be affected severely causing leukopenia, aplastic anemia and agranulocytosis. Affection of other systems e.g. renal, pulmonary and gastrointestinal system may also show evidence of toxic manifestation (Goodman and Gilman, 1996). D-Penicillamine is a costly drug with associated toxic side effects in 20% to 30% of patients.

Therapy with d-penicillamin in a dose of 250 mg thrice daily for 15 days in a group of 5 patients suffering from chronic arsenicosis in West Bengal when followed up for 2-5 years, did not show any difference with control patients (Guha Mazumder et al, 1998d). In contrast, Bansal et al (1991) reported significant improvement in neuropathy of 6 weeks duration after 2 to 4 weeks of D-penicillamine, 750 mg/day even though it was experimentally found to be ineffective in

relieving the systemic symptoms of acute As poisoning (Kreppel et al. 1989). Study on the effect of long term treatment with this agent need to be carried out to ascertain whether such therapy could alter the natural course of chronic As toxicity.

4.3.2. Retinoids

More than 50 years ago, Hall (1946) and colleagues described a beneficial effect of oral supplementation with Vitamin A (retinol) in the treatment of cutaneous arsenicosis. In that report, oral Vitamin A, 150, 000 USP units per day for 3 months resulted in a partial regression of hyperpigmentation and hyperkeratosis of palms in a 39 year old male who had taken Fowler's solution (potassium arsenite) for treatment of childhood chorea. More recently, Thiaprasit (1984) presented a case series of 9 patients with cutaneous arsenicosis who were treated for 2 to 7 months with oral etretinate, a synthetic aromatic retinoid. Clinical and histopathological improvement was noted in arsenical hyperkeratosis, but not in hyperpigmentation. Other case reports of regression of arsenical keratosis with etretinate treatment have been published (Biczko et al, 1986; Sass et al, 1993). It is noteworthy that etretinate and other retinoids have been reported to have antikeratinizing effects in other disorders of keratinization, such as hereditary palmo plantar keratoderma, Pityriasis rubra pilaris, and certain ichthyoses (Fritsch, 1992).

In addition to causing regression in arsenical keratosis, retinoids may offer significant promise in the chemoprevention of As-related cancers. The interaction of endogenous and exogenous retinoids with nuclear receptors influences the expression of genes that effect cell differentiation, proliferation, and induction of apoptosis (Miller, 1998). Some clinical trials, recently reviewed by Lotan (1996) and Hong and Sporn (1997) suggest a beneficial role for retinoids in chemoprevention of cancer in multiple organs. For example, Bouwes Bavinck et al (1995) reported a prospective, double-blind, placebo controlled trial of acitretin in renal transplant patients that resulted in decreased occurrence of cutaneous squamous cell carcinoma and keratotic skin lesions. A prospective randomized, controlled trial of retinoids in patients with chronic cutaneous arsenicosis is clearly indicated at this point of time. However, the therapeutic challenge will lie in selecting the right drug, at the proper dosage, at the correct stage of carcinogenesis. In addition, because retinoids and high dose retinol may have adverse effects, including teratogenesis, such trials will require careful attention to patient selection and surveillance. (Kosnett MJ in press).

4.3.3. Supportive and symptomatic treatment

Though efficacy of specific chelation therapy for patients suffering from chronic As toxicity has not yet been fully substantiated, supportive treatment could help in reducing many symptoms of the patients. Treatment in hospital with good nutritious diet has been found to reduce symptom score in a subset of placebo treated patients in West Bengal during the course of DMSA trial (Guha Mazumder et al 1998c). High protein containing diet, possibly helps in clearance of inorganic As (more toxic) by increased methylation. Thus people should be urged to take food containing proteins in good quantity either from animal source or if unable, from vegetable sources like pulses, soybeans, wheat etc. People should be advised to stop drinking As contaminated water or exposure to As from any other source. Follow up study carried out in West Bengal showed that drinking of As free water did cause improvement of skin manifestations, weakness, anaemia and neuropathy in a significant number of cases (Guha Mazumder et al 1998d). Whether this could decrease the incidence of cancer in the As exposed population is not know.

The various clinical manifestations should be treated symptomatically. Chronic bronchitis with or without obstruction are the common cause of mortality in many cases of chronic As toxicity. It is extremely important that bronchial irritation should be reduced to a minimum. The patient who smokes should be urged to stop completely and permanently. Dusty and smoke laden atmospheres should be avoided. Respiratory infection should be treated promptly because it aggravates breathlessness. Purulent sputum may be treated with oral oxytetracycline or ampicillin in a dose of 250-500 mg 4 times a day or Co-trimoxazole 960 mg twice daily. A 5-10 day course of treatment is usually effective and sputum becomes mucoid. Bronchodilators are much less effective in chronic bronchitis than in bronchial asthma, but should be given to all patients with reversible airflow obstruction. Regular treatment with an inhaled beta₂-adrenoreceptor agonist (Salbutamal 200 mcg or terbutatine 500 mcg, 4-6 hourly) may be sufficient in patients with mild to moderate disease. The anticholinergic bronchodilator drug ipratropium bromide in a dose of 36-72 mcg 6 hourly may be added in patients with more severe air flow obstruction. Theophyllin therapy often has little measurable effect on the airway obstruction associated with chronic bronchitis, but it will improve quality of life in some patients. Treatment option for interstitial lung disease is limited. Dyspeptic symptoms associated with chronic arsenicosis could be easily managed by use of H₂ receptor blockers with/without prokinetic drugs. Though non cirrhotic portal fibrosis occur frequently in these patients, the incidence of portal hypertension is quite low.

When varices are detected by endoscopy prophylactic therapy by betablockers may be of help. Sclerotherapy or banding may be needed for the management of variceal haemohage. Peripheral vascular disease associated with gangrene are difficult to treat because of severe pain. Pharmacological agents like pentoxyphyllin or calcium channel blockers are found to have limited effect. Most of these patients need surgical amputation. Symptoms of peripheral neuropathy improve in some on stoppage of drinking As contaminated water. Tricyclic antidepressants such as amitryptiline may have utility in relieving painful dyesthesias of arsenical peripheral neuropathy (Wilner and Low 1993). Skin thickening of the sole and palm can be treated by local application of keratolytic ointment (Containing 3% salicylic acid) (Saha KC, 1995, Guha Mazumder DN, 1996).

Excision of early skin cancer and bladder cancer due to chronic arsenicosis can be curative. However in advanced cases of those cancers and in cases of internal cancers the treatment options are meager.

4.4. Natural history

Not much information is available in the literature regarding the long-term effect of chronic As toxicity after stoppage of drinking As containing water. Arguello et al (1938) reported that keratoderma appeared insidiously between 2nd and 3rd year of intoxication and didn't disappear after cessation of exposure. Some individuals were followed up for more than 30 years after termination of exposure.

To know the effect of providing safe water to the affected people, a cohort of 24 patients of chronic arsenicosis were re examined after drinking As free water ($As < 10 \mu\text{g/L}$) for a period varying from 2-10 years (13 patients 10 years, 11 patients 2-5 years). These people were drinking As contaminated water (130 to 2000 $\mu\text{g/L}$) earlier for 4 to 15 years. Weakness and anaemia were present in 91.6% and 58.3% of cases initially and was persistent in 60.8% and 33% of cases respectively on repeat examination. Partial improvement of pigmentation and keratosis were observed in 45% and 46% of patients respectively. But liver enlargement was persistent in 86% of cases. However, most distressing observation was new appearance of signs of chronic lung disease (cough, shortness of breath and chest signs) in 41.6% of cases. There was slight reduction of clinical symptoms of neuropathy. It was present in 11 cases (45.8%) at the time of initial examination while in 8 cases (33.8%) during the subsequent period ($P < 0.5$). No new case

of neuropathy was detected in any of the follow-up patients. However, diminished hearing was observed in 5 cases during follow-up examination though it was present in 2 cases initially. Similarly 3 patients complained of dimness of vision during follow-up examination though none had such symptom earlier. None of these three patients had cataract or any other abnormality on fundoscopy. From the above it becomes apparent that not only many of the clinical manifestations of chronic arsenicosis persist for long duration inspite of stoppage of taking As containing water, but new symptoms may appear in some of them. (Guha Mazumder et al. 1998d).

4.5. Outstanding questions and future research needs.

Pigmentation and keratosis are considered diagnostic of chronic As toxicity. However, varied clinical manifestations have been reported to occur in As exposed population. Proper epidemiological study comparing their incidence in As exposed and control population with similar age, sex and socioeconomic status need to be carried out. This will help in identifying specific clinical feature which could be considered diagnostic of chronic As toxicity. It need to be emphasised that many people remain asymptomatic in spite of drinking As contaminated water for many years. Not only there is much variation in the incidence of As related symptoms in an exposed population but only some of an affected family show such features. Goldsmith et al (1980) evaluated the effects of well water As (0.1 to 1.4 mg/l) on health status of residents of Lassen county, California. No particular illness was found to have greater prevalence in groups exposed to elevated As level. Harrington et al (1978) studied exposure level and possible health effect of As in drinking water among residents of a 150 square miles area near Fairbanks, Alaska. The mean concentration of As in water was 0.22 mg/l with some values as high as 2.45 mg/l. No differences were found in signs, symptoms and physical examination findings in the various exposure categories. Valentine et al (1985) surveyed groups of 20 to 57 residents in six United States cities where drinking water concentrations of As ranged from 0.5 to 395 µg/l. No significant difference in the prevalence of gastrointestinal, dermal or neurological symptoms were detected between any of the groups studied. The reasons for non expression of clinical manifestation of chronic As toxicity in many people exposed to prolonged intake of As contaminated water need further study.

Skin lesions are often used as useful precursors to more severe effects like cancer of skin or other internal organs. Cuzick and Co-workers (1984, 1992) observed that palmer keratosis occur early

before people develop As related cancers. However, according to the Technical Panel of the EPA Risk Assessment Forum (USEPA 1988) appearance of such lesions could not be interpreted as a precursor to skin cancer as some malignant skin lesions arise *de novo*. Thus surveillance study of large population exposed to As need to be carried out to ascertain whether As related skin lesions could be used as precursor for cancer of skin or other internal organs.

Chen et al, (1988b) reported that after adjusting for artesian well water consumption, As poisoning (evidence of hyperkeratosis and skin cancer) and undernourishment that a family tendency of Black foot disease persisted and suggested a genetic susceptibility was a sound explanation. Epidemiological study in West Bengal, India, showed that male sex and malnutrition were associated with increased prevalence of skin manifestation in As exposed population. That study further showed that skin lesions occurred in some people with As levels less than 100 µg/L (pigmentation 3.2% & 0.8% and keratosis 1.5% & 0.4% among 274 males and 313 females, respectively, Guha Mazumder et al 1998b). As people in tropical countries like West Bengal, drink water varying from 2 litre to 5 litres per day, significant amount of As exposure occur in these people even with lower level of As in water. Hence for developing a biologically based dose-response model, more studies on individual susceptibility to As need to be carried out after taking into consideration factors like quantity of water taken by people, nutritional status, associated infection, genetic factor etc. Further epidemiological studies with non cancer and cancer end points with low dose of As exposure (0.01-0.05) are also essential to establish safe limit in the population who drink large quantity of water.

Estimation of As level in urine, hair and nail could only give us a corroborative evidence of As exposure either currently or in the recent past. However, high value in these biomarkers can not be solely utilized for the diagnosis of arsenicosis. A better biomarker diagnostic of As related toxicity and carcinogenesis need to be developed.

Genetic bio-marker studies have not only been useful in establishing the link between ingested As and genetic damage, but they are currently being used to provide information into the mechanistic and susceptibility issues of As carcinogenesis as well. Several studies have used one particular genetic biomarker, the micronucleus (MN) assay, to establish the association between drinking water As and genetic damage in the bladder. This assay measures the frequency with which chromosomes and chromosomal fragments are lost to the nucleus during cell division. Studies done on As exposed and unexposed populations in Nevada, Chile, and Mexico have all shown

higher prevalence of MN cells in the urine of exposed subjects compared to unexposed subjects (Warner et al, 1994, Moore et al 1997a, Gonsebatt et al 1997). In one study, an increase in MN cells was seen at urinary As levels of 54 µg/L, a level similar to that attained from drinking water containing 50 µg/L, the permissible upper safe limit of many countries. To further investigate the relationship between As ingestion and MN cells, an intervention study was performed in which the prevalence of these cells in a group of highly exposed Chilean men were measured before and after these men were supplied with water low in As (Moore et al 1997b). After eight weeks of drinking low As containing water, the prevalence of MN cells fell from 2.63 to 1.79 per 1000 cells adding further evidence that ingested As caused genetic injury to the bladder. Despite these findings, it should be emphasized that the relevance of the MN cell biomarker to cancer, as with many genetic biomarkers, remains further to be elucidated.

To develop insight into the actual mechanisms by which As exerts its effects, as well as factors that may determine individual susceptibility to As, other genetic biomarkers are currently being investigated. For example, Steinmaus et al (in press) are currently conducting a bladder cancer case control study in Argentina in which they are collecting oral epithelial cells from cases and controls as a source of DNA for genotype analysis. Two metabolism enzymes, glutathione S-transferase μ (GSTM1) and glutathione S-transferase θ (GSTT1) are important in cancer susceptibility because they may regulate an individual's ability to methylate arsenic (Oya-Ohta et al 1996, Huang et al 1993, Chiou et al, 1997). Carriers of homozygous deletions in these genes (null genotypes) have an absence of enzyme activity and may be more susceptible to potential carcinogens. Hence, the results of this genetic susceptibility analysis can be used to determine if GST genotype influences methylation capabilities and susceptibility to the genotoxic effects of arsenic.

Using lymphocytes from individuals exposed to As, Menzel et al (1997) have searched for a biomarker and mechanism of action of As compounds. AsIII treatment induces a number of proteins as shown by polyacrylamide gel electrophoresis (PAGE). One of the AsIII-induced proteins is heme oxygenase 1 (HO1), an early response enzyme. They have found HO1 was induced in 6 individuals to about the same extent for the same AsIII concentration. A Single dose-response relationship seemed to exist for lymphocyte HO1 content and AsIII dose. Determining HO1 content of circulating lymphocyte for persons exposed to As could provide a biomarker of biological activity. By observing rapid upregulation of proteins like HO1 by AsIII treatment they further conjectured that a nuclear transcription factor might be involved in the

signalling mechanism. Using fresh lymphocytes and lymphoblastoid cells they found that the nuclear transcription factor nuclear factor-kB (NF-kB), but not activator protein 1 (AP-1) was activated by AsIII in a dose-response manner. They propose using the dose-response relationship for activation of lymphocyte NF-kB as a biomarker of the toxic effects of arsenic. Similar dose-response data for Sister Chromatid exchanges/cell (SCEs/cell) can also be used as a biomarker. However, molecular epidemiological studies using various genetic bio-markers need to be carried out to find out whether any of these markers could be used to predict clinical or carcinogenic end point of chronic As exposure in a dose related fashion.

A few reports are available regarding the natural history of chronic As toxicity after the people stop taking As contaminated water. It has been highlighted earlier that though some of the cutaneous and neurological manifestations improve, feature of chronic lung disease appear afresh in new cases. Many of the reports from Taiwan suggest increased incidence of neurological, cardiovascular, cerebrovascular and metabolic (Diabetes mellitus) disease in the previously As exposed population (Chen et al 1997). However individual As exposure data and duration of previous exposure are not available in those reports. Thus to understand the natural history, a well controlled follow up study need to be carried out in an As exposed population having knowledge of individual data regarding dose and duration of As exposure after stoppage of intake of As contaminated water. Any modifying influence of interventions like administration of high protein containing nutritious diet and vitamins need also be studied to find out their efficacy in preventing the occurrence of various non carcinomatous manifestations and development of cancer.

Animal models have demonstrated that various chelating agents like BAL, DMSA and DMPS are efficacious in averting morbidity and mortality if administered within minutes to hours of acute As exposure. In one placebo controlled trial DMSA has not been found to be superior to placebo in altering the clinical score. In a preliminary report DMPS has been shown to have some effect in reducing the symptoms of the treated patients (vide supra). However further study with this agent with a long period of follow up data are still needed before this agent can be advocated for therapeutic use. Limited case series supported by recent insights into the potential mechanisms of As induced carcinogenesis suggest that oral treatment with retinoids (Vitamin A analogues) may have promise in the treatment of chronic cutaneous manifestation of arsenicosis, and may also have impact on the development of neoplasia. Selenium, an antioxidant nutrient that antagonizes many of the effects of As in biological systems, also merits attention as a potential therapeutic

agent for patients with history of chronic As exposure (Kosnett MJ in press). Further studies with retinoids and selenium need to be carried out on As exposed people to ascertain their therapeutic efficacy in modifying the natural history of chronic As toxicity.

4.6. Executive Summary

Chronic arsenic toxicity in man produces a range of clinical manifestations. However, skin manifestations are the most diagnostic. These are characterized by pigmentation of the body and limbs and keratosis of the palms and soles. Rain-drop like spotty pigmentation or depigmentation or diffuse melanosis affecting the whole body are the features of pigmentation. Diffuse thickening of palms and soles with or without nodular elevations are diagnostic of keratosis. Other important clinical features are weakness, anaemia, peripheral neuropathy, liver enlargement, chronic lung disease, and peripheral vascular disease. These features are manifested variably in different exposed populations, and may also be caused by As unrelated conditions. Infrequent manifestations which have also been reported to occur by some investigators in people having history of As exposure and which may also be As unrelated are conjunctivitis, keratitis, rhinitis, cardiovascular disease, nephropathy, and diabetes mellitus. However, many people do not show any such feature despite of drinking arsenic contaminated water for a long time.

Though the various noncarcinomatous manifestations as described above occur in association with chronic arsenicosis, with the exception of skin manifestation others are nonspecific. Hence evidence of chronic As exposure and detection of high levels of As in urine and/or in hair and nails in association with those symptoms need to be considered for the diagnosis of chronic As toxicity. However, normal values in those materials do not exclude the diagnosis of chronic arsenicosis. Presence of specific raindrop pigmentation/depigmentation and keratosis with the history of intake As contaminated water need to be considered as diagnostic hall mark of chronic As toxicity.

Many of the clinical manifestations of chronic As toxicity are irreversible. Epidemiological studies have established As as an important agent which produces cancer of the skin, bladder and lung. Though it produces significant morbidity and occasional fatality, no specific therapy has yet been available. Treatment for chronic As intoxication need to be directed towards a) stoppage of As exposure by providing As free safe water to the exposed population, b) providing specific drug for helping recovery and/or averting disease progression and c) general measures and

symptomatic treatment. Stoppage of intake of As contaminated water and intake of nutritious diets can reduce some of the symptoms of chronic arsenicosis. Whether this could prevent the development of cancer is not known. No specific drug for altering the natural history of the disease has yet been available. However, supportive and symptomatic treatment could help a lot to reduce the suffering of patients.

Arsenic induced skin cancer and bladder cancer could be cured if detected early. Hence a good cancer surveillance programme in chronic As exposed population is essential for preventing cancer related deaths. Mass communication measures should be undertaken highlighting the source of As contamination of drinking water and methodology for obtaining As free safe water in the affected area. People must be desisted from getting panicky. Communication material should aim to debunk myths around As. A belief that arsenicosis is contagious, or similar to leprosy has serious social consequences especially for women and children. The campaign should promote that people sick from arsenic are not a threat to others and need care and attention.

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