Serum levels of malondialdehyde, selenium and arsenic in people living in Nyaung-Done Township, Ayeyawaddy Division

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Abstract

Certain regions in the Ayeyawaddy division in Myanmar have been shown to have high arsenic content in ground water which is the source of drinking water. The aim of the present study was to study the level of serum Malondialdehyde (MDA) and selenium in villagers who might be chronically exposed to arsenic in drinking water. Apparently healthy male and female volunteers (n = 100, male = 46 and female = 54) aged 20 - 40 years who had been using ground water for more than 5 years were recruited. The arsenic content of the drinking water in their wells was found to be 15 - 120 µg/L. Measurement of serum arsenic and serum selenium were done by atomic absorption spectrophotometer. Measurement of blood MDA was done by spectrophotometer. Based on serum arsenic levels, the volunteers were divided into high arsenic group (≥ 0.025 µg/ml) and low arsenic group (< 0.025 µg/ml) ($P < 0.001$). The duration of arsenic exposure of the low serum arsenic group was significantly shorter than those of the high serum arsenic group (8.00 ± 3.69 years vs. 13.85 ± 4.11 years) ($P < 0.001$). There was a significant positive correlation between serum arsenic level and duration of exposure in the study population ($P < 0.001$). There was a significant negative correlation between serum MDA and serum selenium level in the study population ($P < 0.001$). There was no significant difference in mean serum MDA level between low and high serum arsenic groups (1.01 ± 0.21 µmol/L vs. 1.09 ± 0.21 µmol/L; $P > 0.05$). There was significant difference in mean serum selenium levels between low and high serum arsenic groups (43.79 ± 24.44 µg/L vs. 33.66 ± 20.29 µg/L) ($P < 0.05$). The present findings suggest that blood arsenic concentration reflects the duration of exposure to arsenic contaminated water and that the concentration of serum arsenic levels observed in the villagers might be sufficient to induce changes in the plasma levels of selenium, an antioxidant marker, but not in the plasma levels of MDA, an oxidative stress marker.

Introduction

Arsenic is naturally found in surface and groundwater, but its concentration (levels) in the water differ widely depending largely on the underlying geology. Different forms of arsenic exist with varying toxicity to human. The inorganic forms of arsenic, which are the dominant forms in

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surface and groundwater, are the most toxic forms, while the organic forms, common in fish products, are much less toxic[1]. Acute high-dose exposure to arsenic can cause severe systemic toxicity and death. Chronic exposure to lower doses of arsenic can result in subacute toxicity such as skin changes, peripheral sensory and motor neuropathy, diabetes mellitus, peripheral vascular disease, and hepatotoxicity. Long-term effects of arsenic exposure include an increased risk of cancers, even after exposure has ceased[2].

According to data of occupational health in Myanmar, arsenic content of ground water is high in Tha-boung, Kyon-pyaw, Zalun, Hintha-da, Ingapu, Lay-myat-na, Waw, Kyauk-taga, Deik-oo, Kyauk-se, Sit-tway, Kyaungkone and Mazali Township. Since ground water is the primary source of drinking water in this area, many people are chronically exposed to arsenic in drinking water. Although the amounts of arsenic in ground water are too small to cause an immediate health threat, it may cause adverse health effects over a long period of exposure[3].

Oxidative stress is an imbalance between free radical generation and the antioxidant defense system. Wu, Chiou, Wang *et al* (2001) reported that the arsenic concentration in whole blood of individuals is positively associated with the level of reactive oxidants and negatively associated with the antioxidant capacity level in plasma[4]. Selenium is an antioxidant, as a component of the glutathione peroxidase family of enzymes, which protects intracellular structures against oxidative damage[5]. Dietary selenium comes from nuts, cereals, meat, mushrooms, fish, and eggs. Schrauzer (1992) and Anderson and Nielsen (1994) stated that the human body's content of selenium is in the range of 13 - 20 mg and it is known to protect against arsenic toxicity[6,7].

Malondialdehyde (MDA) is a stable product of lipid peroxidation[8]. Lipid peroxidation, the oxidative catabolism of polyunsaturated fatty acid, is widely accepted as a general mechanism for cellular injury and death[9]. Experimental studies have shown that exposure to arsenic results in lipid peroxidation. Arsenic mediated generation of reactive oxygen species involves generation of superoxide (O_2-), singlet oxygen ($1O_2$), the peroxyl radical ($ROO^{\cdot}$), nitric oxide (NO), hydrogen peroxide (H_2O_2), dimethylarsenic peroxyl radicals and also the dimethylarsenic radical.

There may be association between blood arsenic level and the level of MDA (biomarker for lipid peroxidation) and selenium level in people who used arsenic contaminated ground water for drinking and cooking purposes. Therefore, the present study was aimed to study the level of serum MDA and selenium in chronic arsenic exposed subjects living in Mazali village, Nyaung-done Township.

Materials and Methods

Apparently healthy male and female volunteers (n = 100, males = 46 in number and females = 54 in number) aged between 20 - 40 years who were using ground water for more than 5 years (the inclusion criteria) were recruited from Mazali village, Nyaung-done township, Ayeyawaddy division, Myanmar. This research was done in 2012.

Procedure

On the first visit, the volunteers who met the inclusion criteria were chosen by history taking and clinical examination. The subjects were instructed not to eat seafood for 3 days before collection of samples to avoid overestimation of organic arsenic because some fish varieties contain arsenics as arsenobenzene that is non-toxic and mainly excreted in urine.

On the second visit, about 10 ml of venous blood was taken from each subject. Blood samples were collected in metal free test tube and serum was obtained after centrifugation at 1500 rpm for 10 minutes. The samples were analyzed on the day of collection for measurement of MDA level. The serum samples for arsenic and selenium measurements were stored at - 20°C at the Common Research Laboratory of University of Medicine 2, Yangon before being analyzed by atomic absorption spectrophotometer (A.A.S).

Methods

- Measurement of blood arsenic and selenium levels were done by the methods of Pegon (1985)[10] and Oster, and Prellwitz (1982)[11] respectively using the atomic absorption spectrophotometer.
- Measurement of blood MDA level was done spectrophotometrically according to the method of Esterbauer and Cheeseman (1990)[12].

Statistical analysis

Data was analyzed by using the statistical package for Social Sciences (SPSS) software version 13. The values are expressed as mean + SD. Student's "t" test (unpaired) was used to calculate the significance of difference between the means of each parameter (plasma MDA, selenium and arsenic). Pearson's correlation coefficient was calculated to assess the relationship between the continuous variables. Statistical significance level was set at "P" equal to or less than 0.05.

Results

Characteristics of study population

The demographic characteristics of study population were shown in table 1. Based on serum arsenic levels, the volunteers were divided into high arsenic group (≥ 0.025 µg/ml) and low arsenic group (< 0.025 µg/ml) (P < 0.001). Reference value: 0.001 - 0.025 µg/ml[13]. In the low serum arsenic group, 48 % (n = 22) were male subjects and 52 % (n = 24) were female subjects. In the high serum arsenic group, 44 % (n = 24) were male subjects and 56 % (n = 30) were female subjects (table 2).

Comparison of means of duration of arsenic exposure between low and high serum arsenic group

The mean duration of arsenic exposure in low serum arsenic group was 8.00 ± 3.69 years

and that of high serum arsenic groups was 13.85 ± 4.11 years. The duration of arsenic exposure of the low serum arsenic group (n = 46) was significantly lower than that of the high serum arsenic group (n = 54) (P < 0.001) (Figure 1).

Correlation between serum arsenic level and related parameters in the study population

There was a significant positive correlation between serum arsenic level and duration of exposure (P < 0.001) (Figure 2). There were no significant correlations between (1) serum arsenic level and serum MDA level (Figure 3); and (2) between serum arsenic level and serum selenium levels (Figure 4).

Correlation between serum MDA and serum selenium level

There was significant negative correlation between serum MDA and serum selenium levels in the study population (P < 0.001) (Figure 5).

Comparison of means of serum MDA and selenium levels between low and high serum arsenic groups

The mean serum MDA level of low serum arsenic group was 1.01 ± 0.21 µmol/L and that of high serum arsenic group was 1.09 ± 0.21 µmol/L. There difference was not significant (Figure 6). The mean serum selenium level of low serum arsenic group was 43.79 ± 24.44 µg/L and that of high serum arsenic group was 33.66 ± 20.29 µg/L. There was significant difference in mean serum selenium levels between low and high serum arsenic groups (Figure 7).

Table 1. Demographic characteristic of study population

Parameters		Mean ± SD (n = 100)	
Age (years)		28.71 ± 7.08	
		Number	**%**
Sex	Male	46	46 %
	Female	54	54 %
Education	Primary	50	50 %
	Middle School	36	36 %
	High School	10	10 %
	Graduate	4	4 %
Occupation	Casual Workers	26	26 %
	Vendors	23	23 %
	House-wives	23	23 %
	Farmers	18	18 %
	Others	10	10 %

Table 2. Blood arsenic level in study population

Study Population (n)	Low Blood Arsenic Group ≤ 0.025	High Blood Arsenic Group ≥ 0.025
Male	22 (48%)	24 (44%)
Female	24 (52%)	30 (56%)
Total	46 (100%)	54 (100%)

Reference value: 0.001 - 0.025 µg/ml[13]

Figure 1. Comparison of means of duration of arsenic exposure between low (n = 46) and high (n = 54) serum arsenic group
**** indicates significant difference (P < 0.001)**

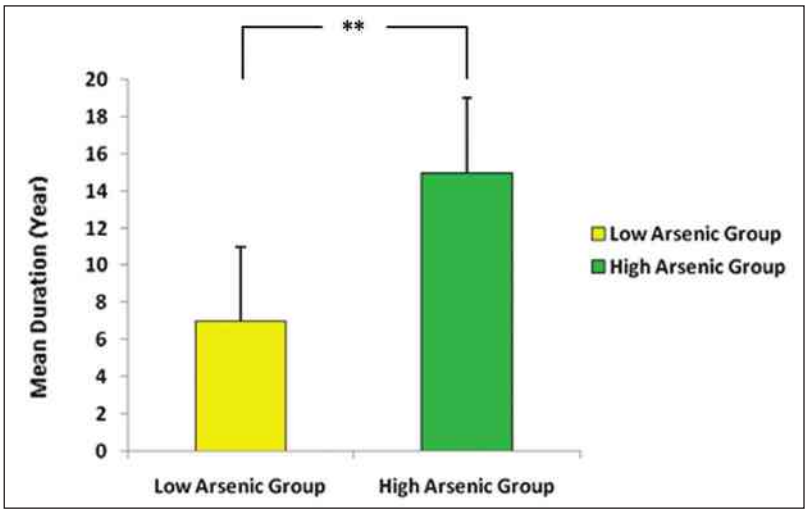


Figure 2. Correlation between duration of exposure to arsenic in drinking water (year) and serum arsenic level

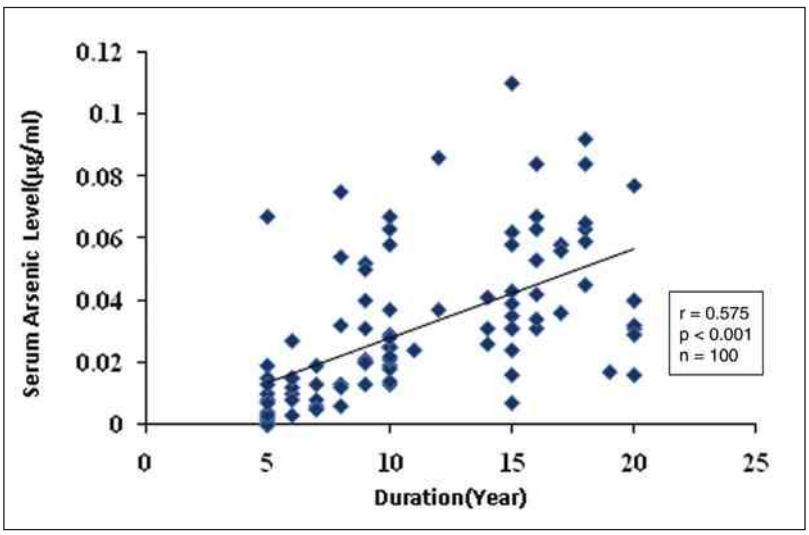


Figure 3. Correlation between serum arsenic level and serum MDA level

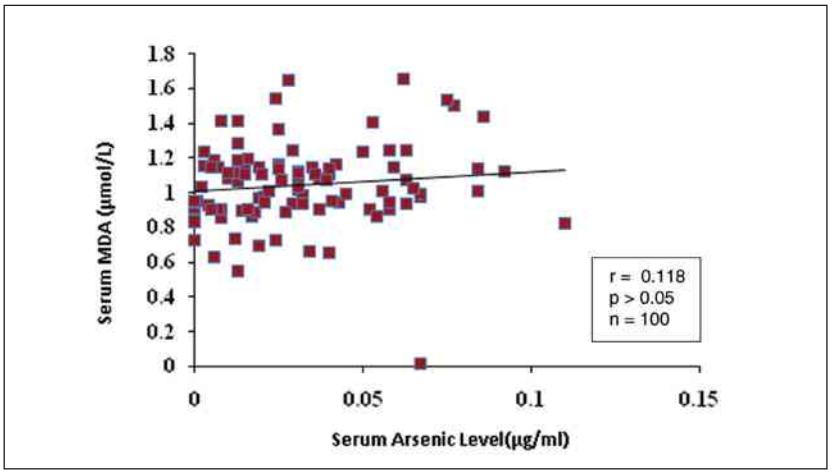


Figure 4. Correlation between serum arsenic level and serum selenium level

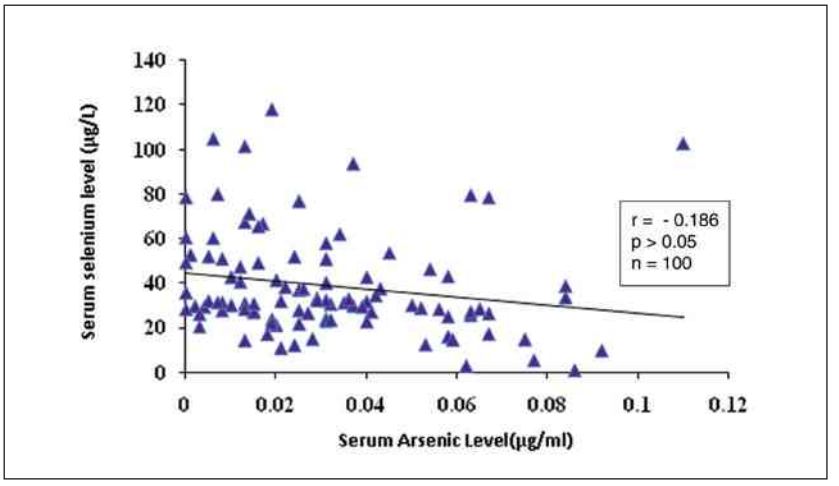


Figure 5. Correlation between serum MDA and serum selenium level

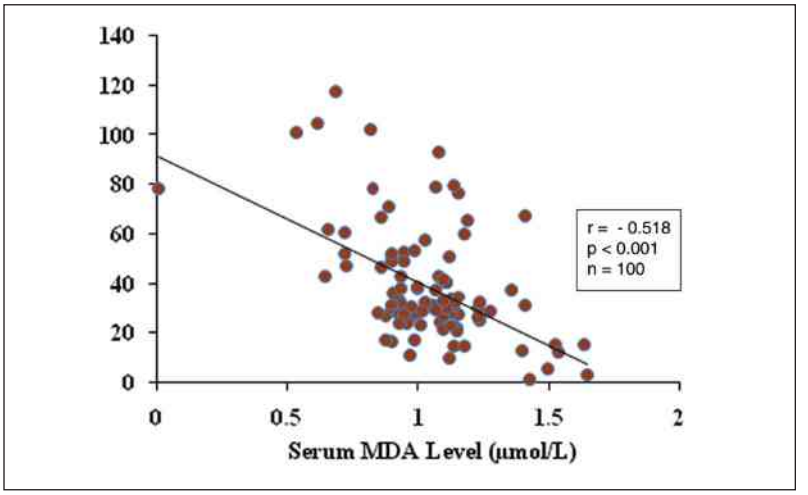


Figure 6. Comparison of mean of serum MDA level (means ± SD) in low (n = 46) and high (n = 54) serum arsenic group
NS - indicates not significant

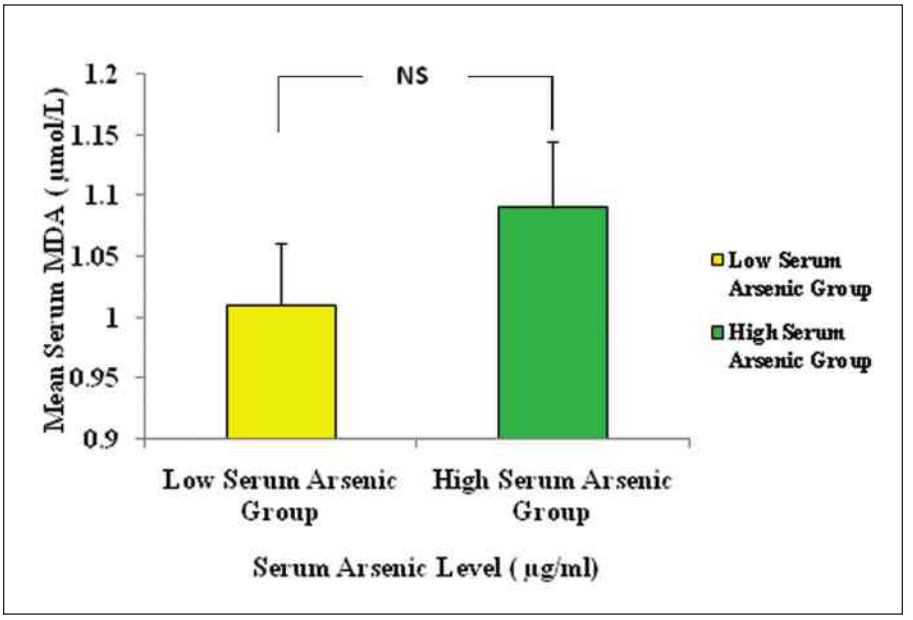
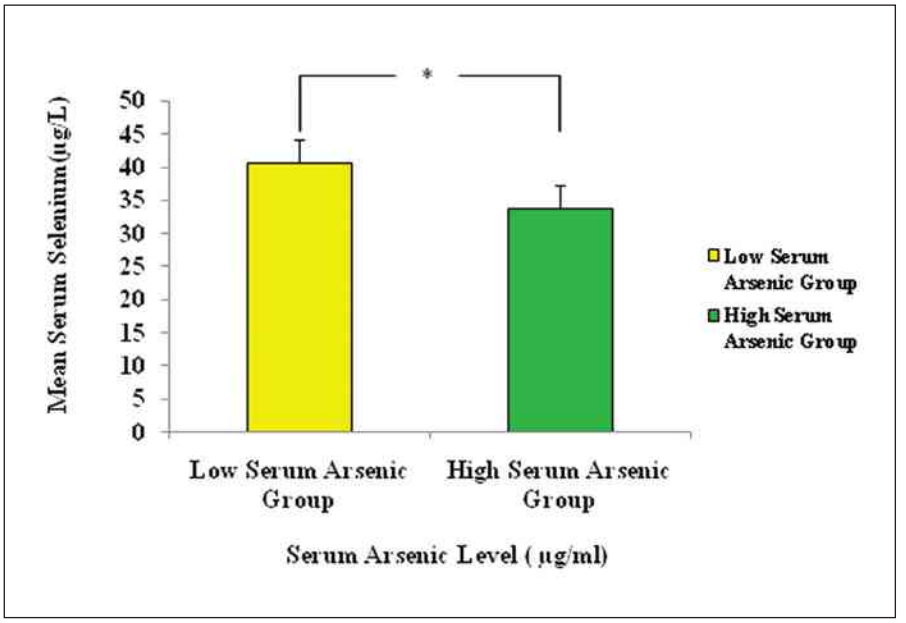


Figure 7. Comparison of mean of serum selenium level (mean ± SD) in low (n = 46) and high (n = 54) serum arsenic groups
*** - indicates P < 0.05**



Discussion

In the present study, the Mazali village, Nyaung-done township, Ayeyawaddy Division was selected for study because most residents have been exposed to a wide range of arsenic levels in water from the artesian well for more than five years. According to the data of occupational health, the arsenic content of water in that village was more than the maximum permissible limit of arsenic in drinking water recommended by World Health Organization which is 0.01 mg/L (10 µg/L)[14]. The present study found that the arsenic content of the drinking water in their wells ranged from 15-120 µg/L.

Serum arsenic level of the study population

Yeoman (1986) reported that the arsenic level in blood in general population was from 0.001 to 0.025 µg/ml. In the present study, the serum arsenic level of 54 % (n = 54) subjects were more than or equal to 0.025 µg/ml and they were regarded as high blood arsenic group; and that of the remaining 46% (n = 46) were less than 0.025 µg/ml and they were regarded as low blood arsenic group.

In 2004, Mandal *et al* reported that the arsenic in drinking water was related to arsenic in the blood. They selected 41 subjects in West Bengal, India and found that the blood arsenic concentration of case subjects (n = 25) who were drinking Arsenic containing water was 0.03 ± 13.8 µg/ml, and that of control subjects (n = 16) was 0.002 ± 6.68 µg/ml. They showed that the serum arsenic level was relatively lower in the control subjects than that of the case subjects ($P < 0.05$)[15].

In 2010, Xue *et al* made a study on all adult residents aged between 18 and 70 years who had been drinking arsenic contaminated water for at least 12 months in Shaunglong Township in China. They found that the serum concentration of arsenic in exposed group group (0.26 ± 0.12 µg/ml) was significantly higher than that in the control group (0.006 ± 0.004 µg/ml) ($P < 0.01$)[16].

In Myanmar, Moe-Moe-Khine (2005) reported that the blood arsenic concentration in case subjects (n = 35) in Zalon Township in Ayeyawaddy Division was significantly higher than that of the control subjects (n = 35) (0.08 ± 0.06 µg/ml vs 0.02 ± 0.02 µg/ml, $P < 0.001$)[17]. The well water arsenic concentration was about 100 - 120 ug/L. May-Su-Nwe and Win-Nwe-Nwe-Thu in 2008 reported similar findings in Kyaungone Township, Ayeyawaddy Division where blood arsenic level of the case group (0.09 ± 0.06 µg/ml) was significantly higher ($P < 0.001$) than that of the control group (0.01 ± 0.001 µg/ml)[18,19].

In the present study, there was a significant positive correlation between serum arsenic level and duration of exposure to arsenic contaminated water ($r = 0.575$, $P < 0.001$). In 2008, May-Su-Nwe also found that there was a significant positive correlation between blood arsenic level and duration of exposure in the study population ($r = 0.575$, $P < 0.001$)[18]. Other studies described that the variations of blood arsenic levels may depend on the duration of exposure to arsenic

contaminated drinking water, the amount of water consumed during the exposed years, the level of nutritional status, socioeconomic status, state of health and their life styles[20].

Serum arsenic and serum MDA level

In the study of Xue *et al* in 2010 in China, the serum MDA level was two times higher in the high arsenic group (24.48 ± 11.47 nmol/ml) than that in the control group (11.01 ± 4.82 nmol/ml), which was statistically significant ($P < 0.001$)[16].

In 2008, Wang *et al* in China made a study in the residents of Xing Reng village. They found that the urinary MDA concentration of the villagers from the arsenicosis endemic area was significantly higher compared to those of the control area. The urinary arsenic concentration in exposed subjects was 192.2 ± 22 μg/ml and that of the control was 63.6 ± 5.9 μg/ml and plasma MDA level in exposed group (n = 113) was 2.5 ± 0.2 μmol/L and that of the control (n = 30) was 1.8 ± 0.3 μmol/L. They reported that there was a strong correlation between urinary arsenic and MDA concentration in this area. Urinary MDA concentration was significantly higher in people with arsenicosis symptoms in endemic areas[21].

In 2006, Liao *et al* stated MDA as an index of lipid peroxidation which can be induced by gallium and arsenic exposure. In their study, the mean urinary arsenic level in exposed group (n = 103) was 32.36 ± 26.87 μg/L and that of the control group (n = 67) was 24.18 ± 22.50 μg/L. The plasma MDA level of exposed group was 0.96 ± 0.46 μmol/L and that of control group was 0.65 ± 0.34 μmol/L ($P < 0.05$). They also found that there was positive correlation of urinary arsenic level and plasma MDA level ($r = 0.265$, $P < 0 .05$)[22].

In the present study, there was no significant correlation between serum arsenic level and serum MDA level in the study population. The mean serum MDA level of high serum arsenic group was 1.09 ± 0.2 μmol/L and that of low serum arsenic group was 1.01 ± 0.21 μmol/L. The serum MDA level of the high serum arsenic group was not significantly higher than that of the low serum arsenic group ($P > 0.05$).

One reason for this could be relatively low serum arsenic levels found in the present study compared to other studies. Nutritional factors (not assessed in the present study) could also play a role as arsenic toxicity can be modified by nutritional status. Diet rich in methyl donar groups (i.e, choline or methionine), selenium and other antioxidants such as vitamin C, and vitamin E could modify arsenic toxicity[20].

Serum arsenic and selenium level

Pilsner *et al* (2010), reported that plasma selenium concentration was inversely associated with total arsenic concentration in blood and urine among 287 adults in Bengladesh. They made a cross-sectional study on adults 18 - 65 years of age who were drinking arsenic contaminated water

with arsenic concentration between 0.1 - 860 µg/L for at least five years. In their study, the mean plasma selenium level was 87.6 ± 1.8 µg/L and that of arsenic concentration in blood was 9.9 ± 6.3 µg/L. They reported that there was a significant negative association with plasma selenium level and blood arsenic level (β = - 0.04; 95% CI, - 0.08 to - 0.01)[23].

In 2007, Chen *et al* evaluated the association between arsenic-related premalignant skin lesions and prediagnostic blood selenium levels in 303 newly diagnosed patients. They reported that the risk of premalignant skin lesions was greater in those with low blood selenium level compared to those with the average level. The findings supported the hypothesis that dietary selenium intake may reduce the incidence of arsenic related premalignant skin lesions among populations exposed to arsenic from drinking water[24].

According to Flora *et al* (1999), the blood arsenic level of the rats treated with gallium arsenide and selenium was significantly lower than that of the groups treated only with gallium arsenide. They concluded that the concurrent selenium treatment may have some preventive value against gallium arsenide at least in reducing the accumulation of arsenic and gallium in major target tissues[25].

In the present study, the serum selenium level of low serum arsenic group was 43.79 ± 24.44 µg/L and that of high serum arsenic group was 33.66 ± 20.29 µg/L. There was a significant difference of mean serum selenium level between low and high serum arsenic group ($P < 0.05$). The present finding was consistent with above findings. Therefore, it might be suggested that dietary selenium intake could influence arsenic toxicity.

However, there was no significant correlation between serum arsenic level and serum selenium level in both low and high serum arsenic groups. It might depend on serum arsenic level, because serum arsenic levels of other studies were markedly higher than the present study. It can be assumed that if the serum arsenic level were as high as in other studies, the correlation between serum arsenic and selenium might be seen.

Arsenic and oxidative stress

Chronic exposure to arsenic from drinking and cooking water in humans results in induction of oxidative stress. Arsenic, mainly arsenite, is a pro-oxidant and reacts with thiols to induce oxidative stress, producing free radical reactions and subsequently apoptosis of cells[26]. Wu *et al* in 2001 found an association of blood arsenic levels with increased reactive oxidants and decreased antioxidants capacity in the residents of Taiwan. They showed that arsenic concentration in whole blood of exposed individuals (9.6 ± 9.9 µg/L) was positively associated with the concentration of reactive oxidants, and negatively associated with the antioxidant capacity of plasma[27].

Xue *et al* (2010) found that there was a significant increase in plasma MDA level in high arsenic group compared with the control group ($P < 0.01$). They also reported a significant negative

correlation between blood arsenic level and the blood selenium level ($r = - 0.555$, $P < 0.001$), and a correlation between high selenium status and elevated activities of serum superoxide dismutase, glutathione peroxidase, catalase and reduced levels of MDA and increased RNA and protein expression of heme oxygenase-1 in peripheral blood mononuclear cells. They suggested that inorganic arsenic exposure is associated with oxidative stress which may be prevented by high selenium status via its antioxidative activity and detoxification effect[16].

In the present study, there was a significant negative correlation between serum MDA and serum selenium levels in the arsenic exposed study population. Oxidative stress results when oxygen free radicals exceed the body's antioxidant defense mechanism. The present study showed that there was an increasing oxidant level and a decreasing antioxidant level to a certain extent.

Conclusion

The present findings show that blood arsenic concentration reflects the duration of exposure to arsenic contaminated water. The concentration of serum arsenic levels observed in the villagers might be sufficient to induce changes in the plasma levels of selenium, an antioxidant marker, but not in the plasma levels of MDA, an oxidative stress marker.

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