Arsenic is a notorious environmental toxicant known as both a carcinogen and an atherogen in human beings, but the pathogenic mechanisms are not completely understood. In cell culture studies, trivalent arsenic enhanced oxidative stress in a variety of mammalian cells, and this association may be closely associated with the development of arsenic-related diseases. To investigate the effect of arsenic exposure on oxidative stress in humans, we conducted a population study to determine the relationships of blood arsenic to reactive oxidants and antioxidant capacity at the individual level. We recruited 64 study subjects ages 42–75 years from residents of the Lanyang Basin on the northeast coast of Taiwan, where arsenic content in well water varies from 0 to ≥3,000 µg/L. We used a chemiluminescence method, with lucigenin as an amplifier for measuring superoxide, to measure the plasma level of reactive oxidants. We used the azino-diethyl-benzthiazoline sulfate method to determine the antioxidant capacity level in plasma of each study subject. We determined arsenic concentration in whole blood by hydride formation with an atomic absorption spectrophotometer. The average arsenic concentration in whole blood of study subjects was 9.60 ± 9.96 µg/L (± SD) with a range from 0 to 46.50 µg/L. The level of arsenic concentration in whole blood of study subjects showed a positive association with the level of active oxidants in plasma (r = +0.41, p = 0.001) and an inverse relationship with the level of plasma antioxidant capacity (r = −0.30, p = 0.014). However, we found no significant association (p = 0.266) between levels of plasma reactive oxidants and antioxidant capacity. Our results also show that the plasma antioxidant capacity levels is lower than the plasma antioxidant capacity levels of in arsenic-exposed individuals. Persistent oxidative stress in peripheral blood may be a mechanism underlying the carcinogenesis and atherosclerosis induced by long-term arsenic exposure. Key words: antioxidant capacity, azino-diethyl-benzthiazoline sulfate method, blood arsenic, chemiluminescence, population study, reactive oxidants, superoxide. Environ Health Perspect 109:1011–1017 (2001). [Online 26 September 2001] http://ehpnet1.niehs.nih.gov/docs/2001/109p1011-1017/au/abstract.html

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Association of Blood Arsenic Levels with Increased Reactive Oxidants and Decreased Antioxidant Capacity in a Human Population of Northeastern Taiwan

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Arsenic, a ubiquitous element present in the environment, is the main constituent of more than 200 mineral species on earth (1). In addition to its natural occurrence in mineral deposits, arsenical compounds are used in many human activities such as manufacturing, agriculture, and medicine (2). Arsenical compounds are transported into the environment mainly by water from wells drilled into arsenic-rich geologic strata or by ambient air during the smelting and burning of coal (1,2). However, the main route of arsenic exposure for the general population is via drinking water (2,3). Epidemiologic studies have documented that long-term exposure to inorganic arsenic (InAs) is associated with an increased risk of cancer of the lung, skin, and probably other anatomic sites (4,5). InAs is also one of major risk factors for blackfoot disease (BFD), a unique peripheral vascular disease identified in endemic areas of arseniasis in Taiwan where residents had used high-arsenic–tainted artesian well water for more than 50 years (6). In addition, cardiovascular disease, such as ischemic heart disease and coronary heart disease (7,8), and cerebrovascular accidents (9) are also closely related to long-term ingestion of high-arsenic drinking water. Arsenic is an unusual environmental toxicant in that it induces carcinogenesis as well as atherosclerosis in human beings. This dual effect of arsenic may itself suggest a common mechanism shared by the pathogenic process of both diseases in their relation to arsenic exposure. A common pathogenic process involving cell proliferation has been suggested for the human carcinogenicity and atherogenicity of long-term exposure to InAs (10). However, the detailed mechanisms by which arsenic causes both cancer and atherosclerotic lesions in humans are not clear. In in vitro studies, arsenite, a trivalent InAs compound, has been shown to induce morphologic transformation (11), structural and numeric chromosome changes (12,13), sister chromatid exchanges (14), gene amplification (15), and micronuclei formation (16,17). Arsenite by itself does not induce gene mutations, but it was shown to potentiate the cytotoxicity, mutagenicity, and clastogenicity of several DNA-damaging compounds (18–20). Barchowsky et al. (21) recently showed that at noncytotoxic concentrations, arsenite increases DNA synthesis in cultured porcine vascular endothelial cells, an indication of a mitogenic response. The detailed mechanisms of arsenic genotoxicity and mitogenicity in cultured cells require further elucidation. Recent studies have indicated that arsenite may interfere with signal transduction pathways either by direct kinase/phosphatase-enzyme inhibition or by redox control of the regulatory molecules (21–24). Furthermore, generation of reactive oxidants during arsenic metabolism can play an important role in arsenic-induced injury (16,25–28). These studies suggest involvement of oxidative stress in the pathogenic effects of arsenic exposure.

Oxidative stress, which results when oxygen free radical generation exceeds the body’s antioxidant defense, has been conventionally considered to have implications in the pathophysiology of several human diseases, including cancer and atherosclerosis (29–31). To investigate the effect of arsenic exposure on oxidative stress at the individual level, we examined reactive oxidants and antioxidant capacity in plasma of an arsenic-exposed yet apparently healthy population, and determined the relationships of the levels of reactive oxidants and antioxidant capacity in...
plasma to the arsenic concentration in whole blood of subjects. We also examined influences of lifestyle characteristics and arsenic metabolism capability on risk associations for any possible confounding effect.

Methods

Study area. In Taiwan, well water with high arsenic levels is clustered in the Lanyang Basin and in the so-called BFD-endemic area in southwestern Taiwan (32). The Lanyang Basin of Ilan County is located on the northeastern coast of Taiwan. The arsenic concentration in well water from the Lanyang Basin area ranges from undetectable to over 3,000 µg/L, and over 50% of surveyed wells contained a level of arsenic below 50 µg/L (33). Although most of the residents in the Lanyang Basin use household-owned well water as their primary drinking source, arsenic-associated cancers observed in the BFD-endemic area of southwestern Taiwan have not yet been found extensively in the Lanyang Basin. However, a high prevalence of cerebrovascular diseases associated with long-term arsenic exposure has recently been reported in the Lanyang Basin (9).

Subject recruitment and blood collection. We focused on two villages—Meicheng and Meifu in Chuangwei Township, Ilan County—where residents currently use household-owned water supply wells as their main drinking source. The total population age 40 years or over in the two villages was approximately 1,000 in 1995. Since then, the population has been regularly followed up for health status (33). The entire population has spent most of their lives in their respective villages (33). The subjects for the present study were recruited from this population.

We first classified the population into four groups according to the arsenic level in their household well water: ≤ 10 µg/L, 10.1–50 µg/L, 50.1–300 µg/L, and > 300 µg/L. Eighty study subjects were grouped by exposure level, each group containing 20 individuals about the same age and equal in sex distribution to those in the other groups. Each subject was scheduled for a health examination in a local hospital. During the hospital visit, each was first asked for consent to join this study. Only study subjects who gave their consent were recruited for blood collection and were given a questionnaire-interview by a nurse in the hospital. All subjects recruited for this study were free of any clinical symptoms such as inflammatory diseases. For each study subject, a 10-mL blood sample was collected into a heparinized and aluminum foil-wrapped blood tube under fasting condition. We stored 3 mL blood sample at −20°C for use to detect arsenic content in whole blood as a measurement of the most recent exposure to arsenic exposure.

The analysis was generally performed within 2 weeks. We centrifuged 2 mL sample to separate plasma from packed cells for subsequent assays of reactive oxidative species and total antioxidant capacity within 6 hr after collection. We interviewed subjects using a formatted questionnaire to obtain relevant information, including demographic and lifestyle characteristics such as cigarette smoking and alcohol and tea consumption, as well as daily sources of drinking water. Only current users of household-owned well water were included in this study. All study subjects were enrolled between November 1997 and May 1998.

Determination of arsenic concentration in whole blood. We determined arsenic content in whole blood for each study subject. We chose blood as the biologic indicator of arsenic exposure to estimate better the relation between arsenic challenge and the resultant oxidative stress for organs and tissues in the body. Once digested as a water solution, arsenic is rapidly transported by the blood to such organs as the liver, kidneys, lungs, intestines, and the skin within 24 hr (34). Although 90% of the bloodborne arsenic is rapidly cleared (34), blood arsenic level is still a useful indicator of continuous arsenic exposure (35). In contrast, urine arsenic is the best indicator of recent exposure of several days, and hair or even fingernail concentrations of arsenic can reflect recent exposure of several months (36). However, these latter two biologic media for determining arsenic exposure suffer a time-lag effect for assessment of their relation to labile radicals formed in study subjects.

To determine the arsenic concentration in whole blood of study subjects, we used a flame atomic absorption spectrophotometric method developed by Wang et al. (37) with slight modification. Briefly, 2 mL of concentrated HNO3 was added to 1 mL of whole blood in a digestion flask. The digestion was maintained at 100°C for 40 min. After addition of 0.7 mL each of concentrated H2SO4 and HClO4, digestion was continued for another 60 min. Finally, 1 mL of high-purity water was added and heated until a colorless solution was obtained. The solution was then diluted to 12 mL and quantified for total arsenic using an atomic absorption spectrophotometer model Z-8000 and its accessory Hydride Formation System HFS-2 (Hitachi, Tokyo, Japan). According to a recovery test for arsenic determination in this study, the recovery of arsenic added to ion-free water was 83.2%, and the interassay variation was 7% (replications = 6).

Measurement of the level of reactive oxidants in plasma. We wrapped heparinized blood samples obtained from study subjects in aluminum foil to prevent light exposure until testing for reactive oxidant levels. To measure the production of oxygen free radicals in samples, we adopted a chemiluminescence method, with slight modification, using lucigenin (1 mg/mL) as an amplifier for measuring superoxide (O2−) (38–40). Briefly, the blood sample was first centrifuged to separate plasma from packed cells, and 80 µL of the plasma was immediately placed in a 96-well dish for oxidative stress assay using a chemiluminescence analyzer (TopCount System; Packard, Meriden, CT, USA). After adding lucigenin, we counted photon emission from the sample at 10-sec intervals at room temperature under atmospheric conditions. In a preliminary experiment, the chemiluminescence level responded immediately after addition of 200 µL lucigenin ([Sigma, St. Louis, MO, USA] 1 mg/mL in phosphate-buffered saline) and approached its maximum at 5 min. Afterward, a steady level lasted for 10 min without a significant change. Thus, the chemiluminescence measurement for each sample was determined at 5 min after addition of lucigenin to the plasma. For each sample, the assay was performed in triplicate, and the reactive oxidant level was expressed as mean chemiluminescence intensity counts (counts per 5 min). All samples were processed in the dark.

Measurement of antioxidant capacity level in plasma. Plasma was separated within 6 hr of collection by centrifugation and preserved at −20°C for antioxidant capacity assay. We measured plasma antioxidant capacity within 1 week using the 2,2’-azinodi[3-ethylbenzthiazoline] sulfonate (ABTS) assay method (Randox Laboratories, Antrim, UK). In this assay, incubation of ABTS with

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**Table 1.** Distribution of age, sex, and arsenic content in blood by arsenic concentration in well water among 64 residents of the Lanyang Basin, northeastern Taiwan, 1997–1998.

| Characteristic                  | Total number | ≤ 10 (n = 15) | 10.1–50 (n = 13) | 50.1–300 (n = 15) | > 300 (n = 21) |
|--------------------------------|--------------|---------------|-----------------|------------------|----------------|
| Mean age in years (range)      | 64           | 56.7 (42–75)  | 58.6 (45–67)    | 60.0 (42–71)     | 54.8 (42–67)  |
| Sex (%)                        |              |               |                 |                  |                |
| Male                           | 26           | 6 (40)        | 5 (62)          | 6 (46)           | 9 (43)         |
| Female                         | 38           | 9 (60)        | 8 (38)          | 9 (60)           | 12 (57)        |
| Mean arsenic content in blood, µg/L (SD) | 64           | 7.2 (8.4)     | 8.1 (12.9)      | 10.8 (10.0)      | 11.4 (9.1)     |
GSTP1 and peripheral plasma antioxidant capacity. We considered two genetic categories encoding enzymes implicated in arsenic metabolism for this study: One involves methyltransferases of arsenic species; the other is the glutathione S-transferase (GST) supergene family. Because sequence information of arsenic methyltransferases is not yet available in the literature, it is impossible to investigate the polymorphisms of these enzymes (41). Instead, arsenic species and their metabolites—including arsenite, arsenate, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)—excercibed in urine are often studied to reflect personal arsenic methylation capability (42). In the present study, we analyzed urinary speciation for each study subject, and the results were published in a previous report (43). To indicate an individual’s primary and secondary methylation capability in arsenic metabolism, we used ratios of MMA to arsenite combined with arsenate (MMA/InAs) and of DMA to MMA (DMA/MMA), respectively.

GSTP1 enzyme has been implicated in arsenic metabolism (44), and the genetic polymorphism at the GSTP1 locus is suggested as an important factor in cancer etiology (45). To determine the genotype at exon 5 of the GSTP1 gene for study subjects, DNA was extracted from samples of peripheral blood cells collected in 1995 (33), and assayed for this study using the polymerase chain reaction–restriction fragment length polymorphism method (46). We first identified two variant GSTP1 cDNAs representing alleles of 105 isoleucine (Ile) and 105 valine (Val) forms of the GSTP1 protein. We then classified study subjects into Ile/Ile, Ile/Val, or Val/Val genotypes. GSTP1 activities are lowest among Val/Val homozygotes, intermediate among Ile/Val heterozygotes, and highest among Ile/Ile homozygotes (46).

Statistical analyses. Each individual oxygen-free radical measurement was logarithm-transformed to stabilize the variance and to cause the distribution to approach normality. All oxygen-free radical values presented in the figures and tables are logarithm-transformed. We used the analysis of variance (ANOVA) F-test to examine the statistical significance of differences in the assayed end points between groups of risk factors. We determined associations between study variables by Pearson’s product-moment correlation coefficients (r). We performed linear regression analysis to examine the effect of arsenic concentration in whole blood on plasma oxidative stress level and total antioxidant capacity after controlling for confounding factors. The level of statistical significance was p < 0.05.

Results

Characteristics of the study population. Table 1 shows the age and sex distribution of study subjects by level of arsenic concentration in well water. We recruited 64 study subjects (80% response rate) for this study. The age of participants ranged from 42 to 75 years, with 26 males and 38 females. The mean and SD of arsenic content in whole blood of each exposure group are also presented in Table 1. People who had used well water with higher arsenic levels had a higher level of arsenic in their whole blood.

The frequency distribution of (logarithm-transformed) plasma reactive oxidant levels and antioxidant capacity levels for study participants are graphed in Figure 1. The plasma reactive oxidants and antioxidant capacity stratified by age, sex, educational level, and main occupation are presented in Table 2. In general, the mean levels of plasma reactive oxidants and antioxidant capacity did not significantly differ among groups stratified by sex, educational level, or main occupation. In contrast, plasma antioxidant capacity decreased with increased age ($\beta$-coefficient = –0.0045, p = 0.014).

Relationship of reactive oxidants to blood arsenic levels. The crude association between levels of plasma reactive oxidants and arsenic concentration in whole blood is illustrated in Figure 2A. The reactive oxidant level was significantly correlated with arsenic concentration in whole blood of study subjects ($r = +0.41$, p = 0.001). The reactive oxidant level increased 0.013 counts/5 min (antilog = 1.03 counts) per 1 µg/L increase in blood arsenic level (p = 0.001). Other study factors, including cigarette smoking and alcohol and tea consumption, as well as three indicators of arsenic metabolism capability—the ratio of MMA to InAs, ratio of DMA to MMA, and GSTP1 genotype—were not significantly (p > 0.05) correlated to the level of plasma reactive oxidants (Table 3).

Previous studies have suggested that oxidative lesions in DNA accumulate with age and are inversely associated with individual antioxidant capacity (29). We thus further adjusted for the aging effect and diminished antioxidant capacity in our regression analyses. As shown in Models I and II of Table 4, the association of reactive oxidant level with arsenic content in whole blood did not change substantially when age and antioxidant capacity were included in the analysis.

Relationship of antioxidant capacity to blood arsenic levels. The distribution of antioxidant capacity levels in relation to arsenic concentration in whole blood is graphed in Figure 2B. Despite the wide variation in individual antioxidant capacity, we observed a significant inverse correlation of plasma antioxidant capacity with arsenic concentration in whole blood ($r = −0.30$, p = 0.014). The plasma antioxidant capacity level decreased 0.0034 mM per 1 µg/L increase in arsenic content in whole blood (p = 0.014) as shown in the $\beta$-coefficient value of the regression line.

As shown in Table 3, there were no significant (p > 0.05) associations of plasma antioxidant capacity with lifestyle characteristics, including cigarette smoking and alcohol and tea consumption. Neither the ratio of DMA to MMA nor the GSTP1 genotype was associated with plasma antioxidant capacity.
capacity in study subjects (p > 0.05). On the other hand, we observed a significant positive association between plasma antioxidant capacity and the ratio of MMA to InAs in urine (p = 0.029). In other words, the lower the primary arsenic methylation capability, the lower the antioxidant capacity level in plasma. However, the decreased level of plasma antioxidant capacity caused by blood arsenic remained significant after adjustment for age and primary arsenic methylation capability (Models III and IV of Table 4).

Discussion

Our data show that 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. The present results, consistent with what we observed in in vitro studies, provide evidence that drinking arsenic-contaminated well water may increase the levels of oxidative stress in peripheral blood in humans. The increased level of reactive oxygen radicals in plasma may represent the net result of increased radical production and decreased antioxidant activity. However, in this study, the positive association between arsenic content in blood and oxygen free radicals in plasma was only partially attributable to the inverse correlation of blood arsenic to plasma antioxidants. These results suggest that arsenic digestion in study subjects enhances the formation of oxygen free radicals in plasma and reduces the antioxidant capacity of arsenic-ingesting subjects.

The free radical theory of arsenic toxicity has recently been gaining acceptance in cell culture studies. Further, the superoxide anion and hydroxyl peroxide (H$_2$O$_2$) are the predominant reactive species produced by cultured cells in response to exposure to arsenite (16,47,48). Our study demonstrates that changes in lucigenin-derived chemiluminescence levels measuring superoxide in plasma were positively associated with arsenic concentration in whole blood of study subjects. These data are consistent with the results of previous studies. However, the detection of superoxide present in plasma of study subjects by the chemiluminescence method may very likely underestimate the levels of reactive oxygen species caused by arsenic ingestion. Nevertheless, the presence of superoxide in plasma is consequential, as shown by its significant association with blood arsenic concentrations in study subjects.

The ABTS method we used in this study to quantify antioxidant capacity uses the ability of test plasma to inhibit the generation of free radicals by metmyoglobin and hydrogen peroxide. Previous studies with cultured cells have shown that several antioxidant activities, such as those of superoxide dismutase (SOD) and catalase, may be modulated by sodium arsenite, thus accumulating superoxide and H$_2$O$_2$, respectively (16,27). In addition to these cellular antioxidant enzymes, nonenzymatic antioxidants, such as glutathione (GSH), bilirubin, ferritin, and uric acid, as well as exogenous antioxidant molecules such as α-tocopherol, β-carotene, and ascorbic acid, also provide primary defense against extracellular and intracellular free radicals (49). In this study, we observed that subjects with higher arsenic content in whole blood had lower antioxidant capacity in plasma. The antioxidants measured in plasma of subjects in this study should represent the components in the extracellular environment, where levels of SOD, catalase, GSH, and GSH peroxidase are often very low (50). Thus, transport or storage proteins, which inactivate the radical generation activities of transition metals by sequestering them, as well as supplementary vitamins may have been the targets for the assay in this study. The individual components of these extracellular antioxidants and the ways the antioxidants are suppressed by ingested arsenic need further elucidation in future studies.

Results of the present study also show that the level of antioxidant capacity in plasma declined with the age of study subjects. The effect of aging on antioxidant activity has been controversial, depending upon the populations studied and antioxidants measured (51–54). In this study, plasma antioxidant capacity was measured by the ABTS method, which assays the total capacity of antioxidant defense. Because antioxidant defense exists as a balanced and coordinated system, the total capacity of the antioxidant defense may give a more precise indication of the relationship between the levels of reactive oxidants and antioxidants (55).

Table 2. Plasma reactive oxidant and antioxidant capacity levels by demographic characteristics among 64 residents of the Lanyang Basin, northeastern Taiwan, 1997–1998.

| Characteristic | Number | Reactive oxidants level Mean ± SD | p-Value | Antioxidant capacity level Mean ± SD | p-Value |
|---------------|--------|---------------------------------|---------|-------------------------------------|---------|
| Total subjects | 64     | 3.001 ± 0.320                   | 1.424 ± 0.112 |                           |         |
| Age groups (years) |        |                                 |         |                                     |         |
| < 50          | 13     | 3.003 ± 0.384                   | 1.473 ± 0.095 |                           |         |
| 50–59         | 25     | 3.091 ± 0.341                   | 1.447 ± 0.092 |                           |         |
| ≥60           | 26     | 2.913 ± 0.244                   | 0.141   | 1.377 ± 0.124                   | 0.016   |
| Sex           |        |                                 |         |                                     |         |
| Male          | 26     | 2.965 ± 0.315                   | 1.428 ± 0.107 |                           |         |
| Female        | 38     | 3.025 ± 0.325                   | 0.467   | 1.417 ± 0.121                   | 0.702   |
| Education level |      |                                 |         |                                     |         |
| Illiterate    | 17     | 3.001 ± 0.300                   | 1.418 ± 0.116 |                           |         |
| Elementary    | 45     | 2.990 ± 0.324                   | 1.428 ± 0.114 |                           |         |
| Junior high and above | 2      | 3.231 ± 0.520                   | 0.589   | 1.393 ± 0.040                   | 0.831   |
| Main occupation |     |                                 |         |                                     |         |
| Retired       | 22     | 2.998 ± 0.316                   | 1.418 ± 0.122 |                           |         |
| Farming       | 28     | 2.923 ± 0.282                   | 1.421 ± 0.122 |                           |         |
| Others        | 14     | 3.149 ± 0.368                   | 0.109   | 1.438 ± 0.077                   | 0.862   |

 Reactive oxidant level (logarithm-transformed) was detected by a chemiluminescence assay measuring the superoxide anion (counts/5 min). Antioxidant capacity level was measured using the ABTS method (mM). Probability derived from an ANOVA F-test for the hypothesis that there is no difference between groups.

Figure 2. Correlations of levels of plasma (A) reactive oxidants (y = 0.013x + 2.8755) and (B) antioxidant capacity (y = −0.0034x + 1.4568) with arsenic concentrations in whole blood among 64 residents of the Lanyang Basin, northeastern Taiwan, 1997–1998. The (logarithm-transformed) reactive oxidant level was detected by a chemiluminescence assay measuring the superoxide anion; r = 0.41, p = 0.001. Antioxidant capacity level was measured using the ABTS method; r = −0.30, p = 0.014.
assayed end point and the risk factors. The total antioxidant capacity of the study subjects in this study decreased 0.0044 mM per yearly increment in age after adjusting for blood arsenic (Table 4). Interestingly, the total antioxidant capacity among Chinese in the geographic area of the present Taiwan study is generally lower than that of a Hong Kong Chinese population assayed in a previous study by other investigators (57). The combined effect of aging and chronic exposure to arsenic of the participants in our study may explain the difference.

We found no significant correlation of lifestyle characteristics, such as cigarette smoking and alcohol and tea consumption, with reactive oxidants level or antioxidant capacity level in plasma. Smoking-related increases in oxidized products including nitric oxides and lipid peroxides have been reported previously (55,56). The nature of the lucigenin-based chemiluminescence assay system for superoxide anion or some unidentified characteristics of study subjects in this study may explain the discrepancy of finding no smoking-related oxidative stress. Ethanol has also been reported to affect oxidative stress level during its metabolism (57,58). Tea components have antioxidant properties and act as free-radical scavengers (59,60). Only a small number of the subjects consumed alcohol or tea in the present study, so random variation due to the small sample size may have occurred.

Table 3. Plasma reactive oxidant and antioxidant capacity levels by lifestyle characteristics and arsenic metabolism capability among 64 residents of the Lanyang Basin, northeastern Taiwan, 1997–1998.

| Characteristic | Number | Reactive oxidant level | Antioxidant capacity level |
|---------------|--------|------------------------|---------------------------|
|               |        | Mean ± SD              | p-Value                   | Mean ± SD              | p-Value                   |
| **Lifestyle characteristic** |        |                        |                           |                          |                           |
| Cigarette smoking | 44     | 2.995 ± 0.345          | 1.432 ± 0.106             |                          |                           |
| Yes           | 20     | 3.013 ± 0.318          | 1.405 ± 0.127             |                          |                           |
| Alcohol consumption | 59    | 3.791 ± 0.320          | 1.428 ± 0.108             |                          |                           |
| Yes           | 5      | 3.390 ± 0.329          | 1.376 ± 0.166             |                          |                           |
| Tea consumption | 58    | 3.794 ± 0.325          | 1.421 ± 0.116             |                          |                           |
| Yes           | 6      | 3.603 ± 0.284          | 1.405 ± 0.085             |                          |                           |
| **Arsenic metabolism capability** |        |                        |                           |                          |                           |
| MMA/InAs ratio | < 1.9  | 3.072 ± 0.424          | 1.380 ± 0.112             |                          |                           |
|                | 21     | 2.507 ± 0.190          | 1.449 ± 0.113             |                          |                           |
|                | ≥ 3.0  | 3.047 ± 0.306          | 1.462 ± 0.077             | 0.029                   |                           |
| DMA/MMA ratio | < 1.9  | 3.034 ± 0.337          | 1.488 ± 0.083             |                          |                           |
|                | 21     | 3.030 ± 0.191          | 1.412 ± 0.109             |                          |                           |
|                | ≥ 3.9  | 2.960 ± 0.408          | 1.412 ± 0.120             | 0.156                   |                           |
| GSTP1 genotype | Ile/Ile | 2.962 ± 0.240          | 1.426 ± 0.112             |                          |                           |
|                | Ile/Val or Val/Val | 3.101 ± 0.450          | 1.407 ± 0.121             | 0.556                   |                           |

*There were two subjects without data on arsenic metabolism capability, and four subjects without data on GSTP1 genotype. Reactive oxidant level (logarithm-transformed) was detected by a chemiluminescence assay measuring the superoxide anion (counts/min). *Antioxidant capacity level was measured using the ABTS method (mM). *Probability derived from an ANOVA F-test for the hypothesis that there is no difference between groups.

Table 4. Multiple linear regression analyses of plasma reactive oxidant and antioxidant capacity levels among 64 residents of the Lanyang Basin, northeastern Taiwan, 1997–1998.

| Model | Variable | Coefficient (× 100) | SEa (× 100) | p-Valueb |
|-------|----------|---------------------|-------------|----------|
| Reactive oxidants levelc | Age (1-year increment) | -0.61 | 0.48 | 0.214 |
|       | Arsenic content in blood (1-µg/L increment) | 1.31 | 0.37 | 0.001 |
|       | Age (1-year increment) | -0.79 | 0.51 | 0.127 |
|       | Total antioxidant capacity (1-mM increment) | -40.77 | 36.33 | 0.266 |
|       | Arsenic content in blood (1-µg/L increment) | 1.17 | 0.39 | 0.004 |
| Antioxidant capacity levelc | Age (1-year increment) | -0.44 | 0.17 | 0.012 |
|       | Arsenic content in blood (1-µg/L increment) | -0.34 | 0.13 | 0.012 |
|       | Age (1-year increment) | -0.35 | 0.16 | 0.037 |
|       | MMA/InAs (< 1.8 vs. 1.8–3.0) | 6.21 | 2.97 | 0.041 |
|       | MMA/InAs (< 1.8 vs. ≥ 3.0) | 7.86 | 2.95 | 0.010 |
|       | Arsenic content in blood (1-µg/L increment) | -0.32 | 0.12 | 0.010 |

*SE of the coefficient, SD/(N-1)1/2; n = 64. *Probability derived from a Wald’s chi-square test for the hypothesis that the regression coefficient = 0. *Reactive oxidant level (logarithm-transformed) was detected by a chemiluminescence assay measuring the superoxide anion (counts/min). *Antioxidant capacity level was measured using the ABTS method (mM).
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damage macromolecules in cells or to act as second messengers, leading to alteration of the gene expression profile in cells and subsequent enhancement of cell proliferation (65, 66). The positive association of reactive oxygen radicals with arsenic content in blood found in this study may explain why arsenic induces both cancers and atheroscerotic lesions at several anatomic sites, as we observed previously among residents of the arseniasis-endemic area (5, 67). Arsenic also reduces antioxidant levels in plasma, which may accelerate disease development at target sites. This contention is consistent with observations of previous studies that levels of β-carotene were lower in patients with arsenic-induced skin cancer (68) as well as in patients with ischemic heart disease (69) than in healthy controls.

In summary, we present evidence that arsenic in blood is not only associated with an increased level of reactive oxygen radicals but is also inversely related to the antioxidant capacity in plasma of humans. The results of this study indicate that arsenic is a significant environmental toxicant that increases the risk of oxidative stress in exposed persons. Persistent high levels of oxidative stress may be a mechanism underlying the carcinogenesis and atherosclerosis induced by long-term arsenic exposure.

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