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Urinary Arsenic Speciation and its Correlation with 8-OHdG in Chinese Residents Exposed to Arsenic Through Coal Burning

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Abstract In contrast to arsenicosis caused by consumption of water contaminated by naturally occurring inorganic arsenic, human exposure to this metalloid through coal burning has been rarely reported. In this study, arsenic speciation and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in urine were determined in the Chinese residents exposed to arsenic through coal burning in Guizhou, China, an epidemic area of chronic arsenic poisoning caused by coal burning. The urinary concentrations of inorganic arsenic (iAs), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and total arsenic (tAs) of high-arsenic exposed subjects were significantly higher than those of low-arsenic exposed residents. A biomarker of oxidative DNA damage, urinary 8-OHdG level was significantly higher in high-arsenic exposed subjects than that of low exposed. Significant positive correlations were found between 8-OHdG levels and concentrations of iAs, MMA, DMA and tAs, respectively. In addition, a significant negative correlation was observed between 8-OHdG levels and the secondary methylation ratio (DMA/(MMA + DMA)). The results suggest that chronic arsenic exposure through burning coal rich in arsenic is associated with oxidative DNA damages, and that secondary methylation capacity is potentially related to the susceptibility of individuals to oxidative DNA damage induced by arsenic exposure through coal burning in domestic living.

Keywords Arsenic · Arsenic speciation · 8-OHdG · Oxidative stress · Coal · Methylation

Arsenic is a naturally occurring element that is ubiquitously present in the environment in both organic and inorganic forms (Nordstrom. 2002; Oremland and Stolz. 2003). Human exposure to the generally more toxic inorganic arsenic (iAs) occurs in occupational or environmental settings, as well as through medicinal arsenical use (Aposhian 1997; Abernathy et al. 1999). The main source of human environmental exposure is through consumption of water containing elevated levels of arsenic, primarily from natural contamination (Nickson et al. 1998; Gebel. 1999). While in China, in addition to arsenicosis induced by the consumption of contaminated drinking water, another type of chronic arsenic poisoning due to burning coal containing high levels of arsenic has been reported (Zheng et al. 1999). Guizhou Province, in the southwest region of China, is the main epidemic area of this type of poisoning. Since coal is plentiful in this area at the surface, burning coal has become the primary fuel source for domestic use, such as cooking, heating and drying of crops. Chronic exposure to high level of iAs through burning coal in domestic living is associated with skin lesions and cancer (Liu et al. 2002).

Accumulating evidence, including our recent studies (Pi et al. 2000, 2002, 2003a, b), indicated that arsenic may induce oxidative stress (Flora et al. 2007), which is linked to the carcinogenetic effect of arsenic (Shi et al. 2004). It has been reported that reactive oxygen species (ROS) can be formed during the process of arsenic methylation

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(Nesnow et al. 2002). Elevated ROS production is associated with increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Tamura et al. 2006), which is associated with the increased risk of cancers (Wu et al. 2004). 8-OHdG has been regarded as one of the most reliable markers of DNA oxidative damage since it reflects extremely low levels of oxidative damage (Wu et al. 2004).

It is generally accepted that iAs is metabolized in humans by enzymatic and non-enzymatic mechanisms into monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which are then rapidly excreted into the urine (Aposhian 1997; Hayakawa et al. 2005). The toxicities of these arsenic species are different. Cytotoxicity assays revealed the following order of toxicity of the arsenic species: trivalent MMA > trivalent iAs > pentavalent iAs > pentavalent MMA = pentavalent DMA (Petrick et al. 2000). Therefore, the individual methylation capacity appears to be meaningful to assess the arsenic toxicity to some extent.

While most cases of arsenicosis are presumed to result from consumption of water contaminated by natural sources of inorganic arsenic, human exposure to arsenic from coal burning is less frequently reported. In this study, we measured urinary arsenic speciation and 8-OHdG levels in the Chinese residents exposed to arsenic through burning coal in domestic living. We aimed to assess the association of individual arsenic methylation capacity of this unique type of arsenic exposure and DNA oxidative damage.

Materials and Methods

The present study was carried out in Jiaole village of Xingren county, southwest of Guizhou Province, China, where coal rich in arsenic is used domestically in daily life for heating, cooking and drying of crops. Arsenic concentrations in coal, stove-dried corn, stove-dried chili and indoor air of Jiaole village were 418 ± 530 mg/kg, 4 ± 3 mg/kg, 512 ± 300 mg/kg, and 0.3 ± 0.2 mg/m^3, respectively (An et al. 2007). Thirty-five volunteer residents (mean age, 45.2 ± 10.2 years; range 36–61 years; 24 males and 11 females) who used coal containing high levels of arsenic for at least 5 years were recruited as high-arsenic exposed subjects. Ten residents (mean age, 40.1 ± 8.1 years; range 31–59 years; 6 males and 4 females) living in the nearby village of Longduo in the same county, where residents used coal containing normal levels, were recruited for the low-arsenic exposed group. Arsenic concentrations in coal, stove-dried corn, stove-dried chili and indoor air of Longduo village were 11.2 ± 2.9 mg/kg, 0.36 ± 0.11 mg/kg, 0.39 ± 0.46 mg/kg, and 0.045 ± 0.041 mg/m^3, respectively (An et al. 2007). Arsenic concentrations in the drinking water of both villages are less than 50 μg/L (National Drinking Water Quality Standard, Ministry of Health, China, 2006).

Although there are 4 nationalities living in the county, all of the subjects recruited are of the Han nationality so as to avoid the possible influence of genetics. The population density of the two villages is similar and residents have the same dietary pattern and habits. Seafood, which is the dietary source of TMA (trimethylarsonic acid) is rare in the diet because of poor economic conditions and long distances from sea. All the subjects who participated in the study provided informed consent. Spot urine samples were collected from all participants, kept on ice, immediately transferred to the Centre for Disease Control and Prevention of Guizhou, and stored at −20°C. Samples then were transported on dry ice to the Laboratory of Arsenic Analysis in China Medical University, where they were finally stored at −80°C until the analysis of urinary arsenic speciation and 8-OHdG levels.

Quantitation of arsenic species were performed as described previously (Pi et al. 2002). Briefly, 1 mL urine that had been stored at −80°C was thawed at room temperature and digested with 2 M NaOH at 95°C for 3 h. The assay sample was stirred with a magnetic stirrer once every 30 min. The treated sample was diluted to 10 mL, and an aliquot sample was used for each assay. iAs and methylated arsenic compounds do not undergo changes in chemical species (e.g. distribution of methyl groups) even when heated in 2 M NaOH (Yamauchi and Yamamura. 1984). iAs, MMA and DMA were determined by atomic absorption spectrophotometer (AA6800, Shimadzu, Japan) with an arsenic speciation pretreatment system (ASA-2SP, Shimadzu, Japan). The detection limit of each of the four chemical species of arsenic (iAs, MMA, DMA, TMA) was 1 ng, and the coefficient of variation was <5%. The standard reference materials used were iAs standard of 1000 mg/L from the National Center for Standard Reference Materials (Beijing, China) and a mixed arsenic standard of 1000 mg/L MMA, DMA and TMA (Tri Chemical Laboratories Inc., Yamanashi, Japan). Quality control for arsenic determinations included the analysis of standard reference material of freeze-dried urine for toxic metals (SRM2670, National Institute of Standards and Technology [NIST], Gaithersburg, MD, USA). The NIST-certified concentration value for arsenic was 480 ± 100 μg/L. The value measured in our laboratory was 474 ± 20 μg/L. Because of limited seafood ingestion, no TMA was detected in the urine sample of any subjects. We reported total arsenic (tAs) concentration by summing the concentration of iAs, MMA and DMA. The final reported urinary arsenic species concentration was adjusted by individual urinary creatinine (Cr) level measured by Jaffe reaction by the method reported previously (Sun et al. 2007). The first and second methylation ratio were

calculated as (MMA + DMA)/tAs and DMA/(MMA + DMA), respectively (Sun et al. 2007).

For the determination of 8-OHdG level, urinary specimens were centrifuged at 15000 rpm for 10 min. Supernatants were then used for the measurement with ELISA Kit (Japan Institute for the Control of Aging, Fukuroi, Japan) according to the method of Saito et al (Saito et al. 2000). The detection range was 0.5–200 ng/mL. The final reported urinary 8-OHdG levels were adjusted by individual urinary Cr concentration.

Data analysis was carried out using SPSS soft ware (version 11.5, SPSS Inc., Chicago, IL, USA). The analysis of arsenic species concentration and 8-OHdG levels were based on logarithmic transformed value. Student's *t-test* was used to compare the differences of urinary arsenic species and 8-OHdG levels between the high-arsenic exposed and low-arsenic exposed groups. Two-way ANOVA were used to evaluate the differences of arsenic species and 8-OHdG levels of female and male between high and low exposed groups. Spearman's correlation was used to analyze the relationship between urinary 8-OHdG levels and various arsenic species, between 8-OHdG levels and the methylation ratios.

Results and Discussion

Arsenicosis has been a major public health problem in China since 1950s (Sun et al. 2001; Sun 2004; Sun et al. 2006). Poisoning caused by long-term exposure to contaminated drinking water has also been found in many other countries all over the world, while chronic arsenic poisoning due to burning coal rich in arsenic is unique to China. Determining the route of exposure from arsenic-containing coal is complicated, as people may be exposed through the ingestion of arsenic contaminated foods (corns and chili peppers) which are dried by burning coal with high concentration of arsenic, as well as through the inhalation of arsenic contaminated air when burning coal for cooking, heating or drying food. Since urinary arsenic is recognized as a reliable indicator of recent exposure to arsenic, it is often used as a biomarker of arsenic exposure (Yamauchi and Fowler 1994). In the present study, we measured the concentrations of urinary arsenic species in 45 subjects, calculated tAs by summing the levels of iAs, MMA and DMA for each individual, and regarded tAs as the individual exposure level to arsenic. We found that the urinary arsenic concentrations of iAs, MMA, DMA and tAs of the high-exposed group were significantly higher than those of low-exposed group (Table 1); when subjects were divided into male and female, we also found that the urinary concentrations of iAs, MMA, DMA and tAs in males, and iAs, DMA and tAs in females exposed to

Table 1 Increased urinary levels of arsenic species and 8-OHdG in subjects exposed to arsenic by burning coal in domestic living

	Low geometric mean (95% confidence interval)			High geometric mean (95% confidence interval)		
	Male ($n = 6$)	Female ($n = 4$)	Total ($n = 10$)	Male ($n = 24$)	Female ($n = 11$)	Total ($n = 35$)
iAs (μg/g Cr)	2.67 (0.01, 4.64)	2.92 (1.32, 7.00)	2.77 (0.35, 5.18)	6.81* (4.51, 9.11)	8.52* (4.23, 10.02)	7.31** (5.04, 9.57)
MMA (μg/g Cr)	2.70 (0.23, 5.16)	3.80 (0.93, 6.68)	3.10 (0.70, 5.49)	7.83* (5.57, 10.08)	6.98 (4.13, 9.83)	7.55** (5.28, 9.83)
DMA (μg/g Cr)	16.90 (14.43, 19.38)	17.56 (14.41, 20.71)	17.16 (14.72, 19.60)	43.72* (41.56, 45.88)	38.04* (35.49, 40.59)	41.85** (39.67, 44.03)
tAs (μg/g Cr)	22.54 (20.09, 24.99)	24.63 (21.55, 27.71)	23.35 (20.94, 25.77)	59.39* (57.21, 61.57)	56.12* (53.54, 58.69)	58.34** (56.15, 60.53)
8-OHdG (mg/g Cr)	5.38 (2.18, 8.59)	7.62 (4.56, 10.68)	6.19 (3.45, 8.92)	14.49* (12.34, 16.63)	16.47* (14.26, 18.67)	15.08* (12.98, 17.19)

Low, low-arsenic exposed; high, high-arsenic exposed

* $p < 0.05$ vs. low-arsenic exposed subjects; ** $p < 0.01$ vs. low-arsenic exposed subjects

arsenic-rich coal were markedly higher than those measured in the low-exposed group (Table 1). The correlation between urinary arsenic levels and arsenic content in coal provide strong evidence that coal burning is an important source of arsenic exposure in Jiaole village.

It has been reported that arsenic exposure is associated with the production of ROS that result in oxidative damage (Huang et al. 2004; Shi et al. 2004). Our previous studies also revealed a correlation between arsenic exposure and oxidative stress in cultured cells (Pi et al. 2003b), experimental animals (Pi et al. 2003a), and epidemiological investigations from arsenicosis caused by consumption of contaminated drinking water (Pi et al. 2000, 2002). Arsenic is a confirmed human carcinogen. Besides cancers such as skin and lung, induced by arsenic exposure through drinking water (Sun et al. 2006), it has also been reported that arsenic exposure through coal burning can induce skin cancers (Liu et al. 2002). Since oxidative stress is involved in many stages that regulate cancer development (Karihtala and Soini. 2007), it has been proposed that the production of ROS induced by arsenic exposure is one of the possible mechanisms of arsenic-related carcinogenesis (Shi et al. 2004). Among the macromolecular species produced by ROS stress, 8-OHdG, an oxidized nucleoside of DNA, is not only a biomarker of generalized, cellular oxidative stress but might also be a risk factor for cancer. Elevated levels of 8-OHdG has been reported to be linked with increased risk of many cancers (Wu et al. 2004). In this study, we detected 8-OHdG levels in the populations exposed to arsenic through domestic coal burning to evaluate the relationship between arsenic exposure and urinary 8-OHdG levels. We found that the 8-OHdG levels in urine from male subjects and female subjects of high-exposed group were significantly higher than those of

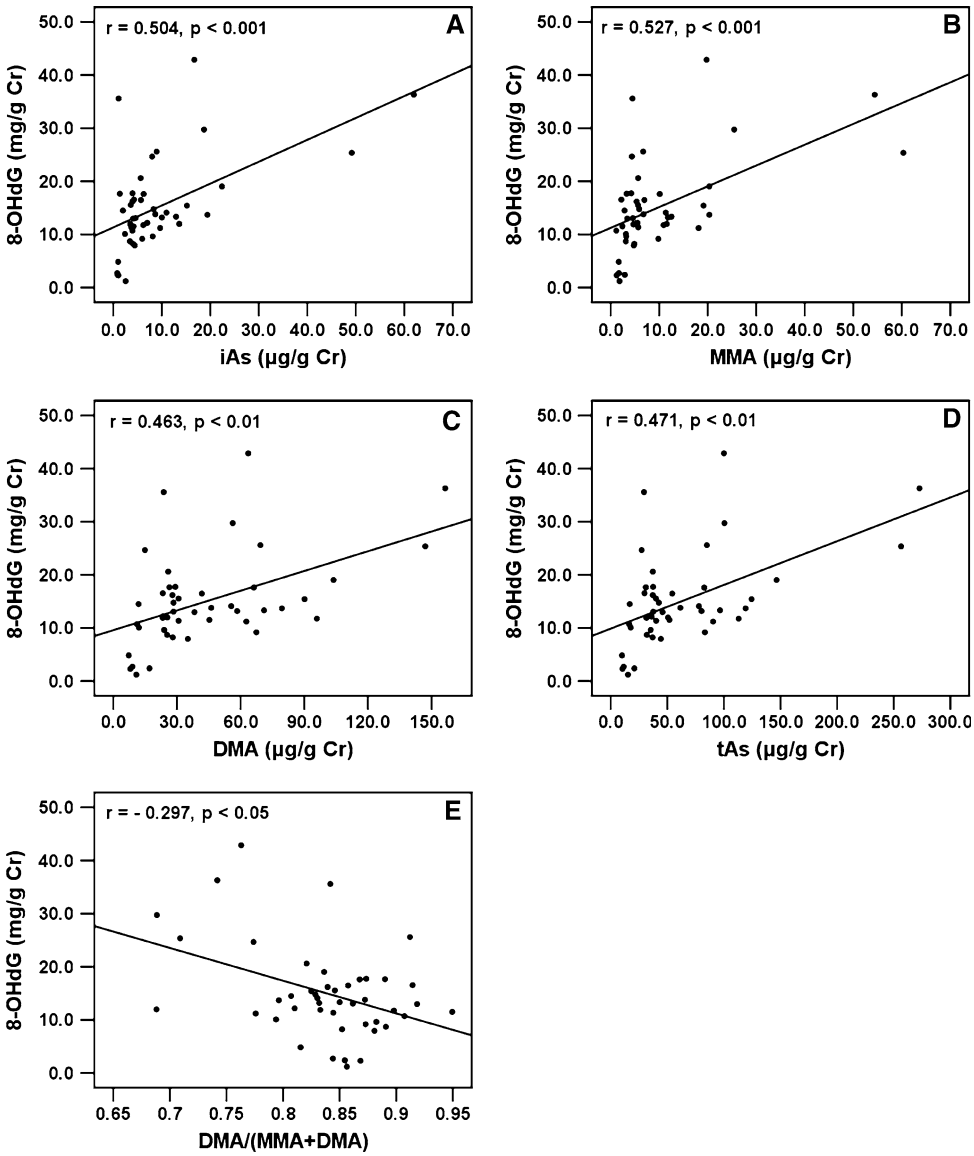

Fig. 1 Correlation of urinary 8-OHdG with (**A**) iAs ($r = 0.504$, $p < 0.001$); (**B**) MMA ($r = 0.527$, $p < 0.001$); (**C**) DMA ($r = 0.463$, $p < 0.01$); (**D**) tAs ($r = 0.471$, $p < 0.01$); (**E**) DMA/(MMA + DMA) ($r = -0.297$, $p < 0.05$). Fit line was added for the significant correlation

low-exposed (Table 1), which provided evidence that chronic arsenic exposure through burning coal containing high level of arsenic is related with oxidative DNA damage. In addition, the urinary 8-OHdG levels were also found to be significantly correlated with the concentration of iAs, MMA, DMA and tAs, with positive Spearman's correlation coefficients of 0.504 (Fig. 1A), 0.527 (Fig. 1B), 0.463 (Fig. 1C) and 0.471 (Fig. 1D). This result suggested that arsenic exposure through coal burning may dose-dependently induce oxidative DNA damage.

Since the toxicity of arsenic species are different from each other, with the trivalent forms being more toxic than the pentavalent forms, and MMA being more toxic than DMA (Styblo et al. 2000; Petrick et al. 2000), the individual methylation capacity seems to be meaningful to assess the arsenic toxicity to some extent. As we know, iAs is metabolized into MMA by the first methylation, and MMA is then metabolized into DMA by the second methylation. MMA is both the product of the first methylation of iAs and the substrate of the second methylation that produces DMA. Therefore, as far as the first methylation ratio is concerned, the parts of MMA that have been metabolized into DMA should be included in the numerator, while the parts of iAs that have been metabolized into MMA and DMA should be included in the denominator, thus, the first methylation ration should be calculated as (MMA + DMA)/tAs. As for the second methylation ratio, the parts of DMA which is produced from MMA should be included in the denominator, hence, the second methylation ratio should be calculated as DMA/(MMA + DMA). Since methylation capacity is a meaningful index to assess the arsenic toxicity, we also assessed the relationship between 8-OHdG level and methylation ratios. A significant negative correlation was found between 8-OHdG level and secondary methylation ratio as shown in Fig. 1E with Spearman's correlation coefficients of -0.297 ($p < 0.05$). These results suggest that secondary methylation of toxic product, MMA to a less toxic form, DMA represents an important detoxification pathway. Furthermore, the reduced cytotoxicity of DMA may be associated with reduced levels of 8-OHdG. Taken together, these results indicate that arsenic exposure through burning coal rich in arsenic is associated with oxidative DNA damage, and individual secondary methylation capacity can be an important susceptibility factor for oxidative DNA damage induced by arsenic exposure from domestic coal burning.

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References

Abernathy CO, Liu YP, Longfellow D, Aposhian HV, Beck B, Fowler B, Goyer R, Menzer R, Rossman T, Thompson C, Waalkes M (1999) Arsenic: health effects, mechanisms of actions, and research issues. Environ Health Perspect 107(7):593–597. doi:10.2307/3434403

An D, Li DS, Liang Y, Jing ZJ (2007) Unventilated indoor coal-fired stoves in Guizhou province, China: reduction of arsenic exposure through behavior changes resulting from mitigation and health education in populations with arsenicosis. Environ Health Perspect 115:659–662

Aposhian HV (1997) Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu Rev Pharmacol Toxicol 37:397–419. doi:10.1146/annurev.pharmtox.37.1.397

Flora SJ, Bhadauria S, Kannan GM, Singh N (2007) Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: a review. J Environ Biol 28(2 Suppl):333–347

Gebel TW (1999) Arsenic and drinking water contamination. Science 283(5407):1458–1459. doi:10.1126/science.283.5407.1455e

Hayakawa T, Kobayashi Y, Cui X, Hirano S (2005) A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. Arch Toxicol 79:183–191. doi:10.1007/s00204-004-0620-x

Huang C, Ke Q, Costa M, Shi X (2004) Molecular mechanisms of arsenic carcinogenesis. Mol Cell Biochem 255(1–2):57–66. doi:10.1023/B:MCBI.0000007261.04684.78

Karihtala P, Soini Y (2007) Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. APMIS 115(2):81–103. doi:10.1111/j.1600-0463.2007.apm_514.x

Liu J, Zheng B, Aposhian HV, Zhou Y, Chen ML, Zhang A, Waalkes MP (2002) Chronic arsenic poisoning from burning high-arsenic-containing coal in Guizhou, China. Environ Health Perspect 110(2):119–122

Nesnow S, Roop BC, Lambert G, Kadiiska M, Mason RP, Cullen WR, Mass MJ (2002) DNA damage induced by methylated trivalent arsenicals is mediated by reactive oxygen species. Chem Res Toxicol 15(12):1627–1634. doi:10.1021/tx025598y

Nickson R, McArthur J, Burgess W, Ahmed KM, Ravenscroft P, Rahman M (1998) Arsenic poisoning of Bangladesh groundwater. Nature 395(6700):338. doi:10.1038/26387

Nordstrom DK (2002) Public health. Worldwide occurrences of arsenic in ground water. Science 296(5576):2143–2145. doi:10.1126/science.1072375

Oremland RS, Stolz JF (2003) The ecology of arsenic. Science 300(5621):939–944. doi:10.1126/science.1081903

Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Aposhian HV (2000) Monomethylarsonous acid (MMA^{III}) is more toxic than arsenite in Chang human hepatocytes. Toxicol Appl Pharmacol 163:203–207. doi:10.1006/taap.1999.8872

Pi J, Kumagai Y, Sun G, Yamauchi H, Yoshida T, Iso H, Endo A, Yu L, Yuki K, Miyauchi T, Shimojo N (2000) Decreased serum concentrations of nitric oxide metabolites among Chinese in an endemic area of chronic arsenic poisoning in Inner Mongolia. Free Radic Biol Med 28(7):1137–1142. doi:10.1016/S0891-5849(00)00209-4

Pi J, Yamauchi H, Kumagai Y, sun G, Yoshida T, Aikawa H, Hopenhayn-Rich C, Shimojo N (2002) Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water Environ Health Perspect 110:331–336

Pi J, Horiguchi S, Sun Y, Nikaido M, Shimojo N, Hayashi T, Yamauchi H, Itoh K, Yamamoto M, Sun G, Waalkes MP, Kumagai Y (2003a) A potential mechanism for the impairment

of nitric oxide formation caused by prolonged oral exposure to arsenate in rabbits. Free Radic Biol Med 35(1):102–113. doi:10.1016/S0891-5849(03)00269-7

Pi J, Qu W, Reece JM, Kumagai Y, Waalkes MP (2003b) Transcription factor Nrf2 activation by inorganic arsenic in cultured keratinocytes: involvement of hydrogen peroxide. Exp Cell Res 290:234–245. doi:10.1016/S0014-4827(03)00341-0

Saito S, Yamauchi H, Hasui Y, Kurashige J, ochi H, Yoshida K (2000) Quantitative determination of urinary 8-hydroxydeoxyguanosine (8-OH-dg) by using ELISA. Res Commun Mol Pathol Pharmacol 107:39–44

Shi H, Shi X, Liu KJ (2004) Oxidative mechanism of arsenic toxicity and carcinogenesis. Mol Cell Biochem 255(1–2):67–78. doi:10.1023/B:MCBI.0000007262.26044.e8

Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas DJ (2000) Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch Toxicol 74(6):289–99. doi:10.1007/s002040000134

Sun G, Liu S, Li B, Li X, Sun X, Guo X, Qian C, Pi J (2001) Current situation of endemic arsenicosis in China. Environ Sci 5:425–434

Sun G (2004) Arsenic contamination and arsenicosis in China. Toxicol Appl Pharmacol 198(3):268–271. doi:10.1016/j.taap.2003.10.017

Sun G, Li X, Pi J, Sun Y, Li B, Jin Y, Xu Y (2006) Current research problems of chronic arsenicosis in China. J Health Popul Nutr 24(2):176–181

Sun G, Xu YY, Li X, Jin Y, Li B, Sun X (2007) Urinary arsenic metabolites in children and adults exposed to arsenic in drinking water in Inner Mongolia, China. Environ Health Perspect 115:648–652

Tamura S, Tsukahara H, Ueno M, Maeda M, Kawakami H, Sekine K, Mayumi M (2006) Evaluation of a urinary multi-parameter biomarker set for oxidative stress in children, adolescents and young adults. Free Radic Res 40(11):1198–1205. doi:10.1080/10715760600895191

Wu LL, Chiou CC, Chang PY, Wu JT (2004) Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 339(1–2):1–9. doi:10.1016/j.cccn.2003.09.010

Yamauchi H, Fowler BA (1994) Toxicity and metabolism of inorganic and methylated arsenicals. In: As in environment. part II: human health and ecosystem effects. Wiley, New York, p 35–43

Yamauchi H, Yamamura Y (1984) Metabolism and excretion of orally administered dimethylarsinic acid in the hamster. Toxicol Appl Pharmacol 174:134–140. doi:10.1016/0041-008X(84)90279-5

Zheng B, Ding Z, Huang R, Zhu J, Yu X, Wang A, Zhou D, Mao D, Su H (1999) Issues of health and disease relating to coal use in southwestern China. Int J Coal Geol 40:119–132. doi:10.1016/S0166-5162(98)00064-0