Toxicology Research

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/toxicology

The combine effects of fluorine and arsenic on renal function in Chinese population

Qi-bing Zeng, [a] Yu-yan Xu, [a] Xian Yu, [b] Jun Yang, [b] Feng Hong, [*a] Ai-hua Zhang, [a]

[a] Guiyang Medical University, School of Public Health, 9 Beijing Road, Guiyang 550004, P.R. China.
[b] Guiyang Centers for Diseases Control and Prevention, Guiyang 550001, P.R. China.
* Corresponding author
Telephone: +86 851 6909 908
Fax: +86 851 6908 908
E-mail: 178945324@qq.com

Abstract

Chronic exposure to combined fluoride and arsenic continues to be a major public health problem worldwide, affecting thousands people. Although there have been reports in literature of individual toxicity of arsenic and fluoride, however, there is very little known about the combined effects of these two elements. In this study, the fluoride-arsenic exposed population as the subject, observation the changes of renal function biomarkers after exposure to different concentrations of fluoride and arsenic. The results show that fluoride and arsenic can induced the glomerular and tubular injury. In this exposure levels, the combined effect of fluoride and arsenic on renal function is mainly antagonism. Moreover, the benchmark dose and its lower confidence limit were calculated to estimate the exposure limit of urine arsenic (UAs) and urine fluoride (UF). The results indicate that the critical concentrations of UF and UAs for renal function injury were estimated as 0.54mg/gCr and 10.50μg/gCr in population co-exposed to fluoride and arsenic, respectively; and urine albumin can be used as a relative sensitive biomarker of renal function.

Keywords: fluoride; arsenic; renal function.

Introduction

Fluorine is an essential trace element in human bodies. Arsenic is an environmental toxicant and a known carcinogen. Both arsenic and fluoride are ubiquitous in the environment. Chronic exposure to combined fluoride and arsenic continues to be a major public health problem worldwide, affecting thousands people.[1] Many studies have reported simple fluorosis and

arsenicosis, however, there is very little known about the combined effect of these two elements. Few contradictory results were reported in experimental studies in which different joint actions such as independent, synergistic and antagonistic effects were observed.[2-3] This indicates that interaction mechanism of these two elements is considerable complicated. Therefore, it is very necessary to conduct in-depth research and to better understand the joint action of these two toxicants.

Arsenic is 33rd element in the periodic table. The possible methods of exposure to arsenic are contact, ingestion and inhalation. Once absorbed, arsenic rapidly combines with the globin portion of hemoglobin, then distributed in other organs, such as liver, kidney, spleen, lung and gastrointestinal tract, muscle and nervous tissue.[4-5] Arsenic is cleared from the body relatively rapidly and primarily through kidney. These processes will produce toxic effects on the kidney. Some epidemiological and animal experimental results confirmed the renal toxicity of arsenic.[6-8] Fluorine is the 13th most abundant element on earth. Inorganic fluorides are almost completely absorbed from the gastrointestinal tract by a process of simple diffusion. Then, it is rapidly distributed from plasma to all tissues and organs. Fluoride mainly excreted by the kidney. The kidney has a higher concentration than plasma, because fluoride is concentrated to high levels within the kidney tubules.[9-10] Thus, the kidney vulnerable to the hazards of fluorine. Recent studies have shown that fluoride induced renal cell apoptosis and tubular function.[11-12] Currently, some studies have reported the simple fluoride and arsenic effects on renal, however, there is very little known about the combined effect of these two elements on renal.

Toxicology research and toxicity testing constitute the scientific core of an important activity known as risk assessment, which is used for the evaluation of potential adverse health impacts from chemical exposures. Risk assessment, which is based on toxicological test data to determine an acceptable level or safe dose. Currently, the risk assessment is primarily based on observational data, exposure to fluorine and arsenic alone, and did not take into account the interaction between the fluorine and arsenic. Therefore, it is necessary to establish exposure limits in population who co-exposure to fluorine and arsenic.

In this study, the fluoride-arsenic exposed population as the study subject, by observing the changes of renal function biomarkers after exposure to different concentrations of fluoride and arsenic, we aim is to explore combined effect of fluoride and arsenic on renal function. Moreover,

the study was designed to explore the critical concentrations of urinary fluoride (UF) and urinary arsenic (UAs) for renal function damage in Chinese population who co-exposure to fluorine and arsenic.

Materials and Methods

Study Population

This is a population-based study; the selected investigation site was located in Liuchang village, Qinzhen city, Guizhou province, China. Guizhou province is one of the serious region of endemic disease in china. A baseline survey of 45,364 residents in 2004 identified more than 2,800 individuals with arsenicosis.[13-14] Currently, Guizhou province have about 34.37 million people at risk exposure to fluoride，including about 16.2 million fluoride dental patients and more than 1.86 million patients with skeletal fluorosis.[15] Written informed consent was obtained from all participants. The study proposal was reviewed and approved by the Ethical Committee of Guiyang Medical University. We worked with Guiyang Centers for Diseases Control and Prevention and its subordinate units to recruit volunteers. All participants were required to be permanent residents of the local area（Liuchang village）and were matched for age and sex. The total number of participants was 196. Exclusion criteria included smoking, drinking, pregnancy, family history of high cancer incidence and recent history of taking seafood and drug, which could affect renal function, urinary excretion of arsenic and fluoride.

The ACGIH biological exposure index is 35 μg /L for arsenic and 2 mg/L for fluoride in urine. In the survey area, the average of creatinine in urine was 1.7mg/L in the normal population. After standardized to the average of creatinine (1.7mg/L), the biological exposure index is about 20μg/g creatinine for arsenic and 1.2mg/g creatinine for fluoride in urine. Based on the reference values recommended by arsenic and fluorine in urine, with double geometric spacing, urine fluoride (UF) was divided into control (<1.2mg/gCr), low (1.2~2.4mg/gCr), high (>2.4mg/gCr) three levels, and urine arsenic (UAs) was divided into control (<20μg/gCr), low (20~40μg/gCr), high (>40μg/gCr) three levels.

Collection of Samples and Analytical Method

After giving informed consent, fasting venous blood samples and morning urine samples were collected. All participants had been instructed on how to avoid contamination. All samples were

stored at -20℃ until analysis. 1 ml of urine sample was acidified with hydrochloric acid and was used for UAs assay. The rest of urine sample was used for the measurement of UF , urinary albumin(UALB), urinary N-acety1-beta-D-glucosaminidase (UNAG), urinary Alpha-1-micro-globulin (Uα1-M G) and urinary creatinine（UCr）levels. All urinary parameters were standardized to the concentration of creatinine in urine. UF was determined by fluorine ion selective electrode method (PXJ-1B digital ion meter) which is for the national health industry standard method in China, the accuracy of 96.5%～102.5%, coefficient of variation (precision) of 0.5%～3.7%. UAs was determined by atomic fluorescence spectrometry method (AF-610D2 chromatography-atomic fluorescence spectrometry) which is for the national health industry standard method in China, the accuracy of 97.5%～113.6%, coefficient of variation (precision) of 7.0%～9.1%. The concentration of UALB, UNAG and Uα1-MG were determined by enzyme linked immunosorbent assay method. The concentration of UCr was determined by the Jaffe reaction method (T6 UV-visible spectrophotometer). These biomarkers were determined using kits from the Nanjing Jiancheng Biological Engineering Institute in China.

Statistical Analysis

SPSS version 13.0 software were used for frequency, partial correlation, χ^2 tests for trends, F test and covariates for regression models analysis. For comparisons between more than two groups, a one-way analysis of variance was used. Distributions of the biological measurements were normalized by logarithmic transformation. The data were expressed in terms of geometric means and their 95% confidence interval. Benchmark dose was calculated using a U.S. EPA BMDS version 2.4.0 software.

Results

The results of the epidemiological investigation

The content of fluoride and arsenic in environmental samples: This area is a fluorine pollution ward, the results show that fluorine content reach about 49.27～1633.42mg/kg in clay; 23.07～474.34mg/kg in coal. It was shown that the average fluorine content in an ambient air was 0.03 mg/m^3, 1.46 mg/kg in rice, 1.47mg/kg in corn and 14.29 mg/kg in pepper because of burning coal contain fluorine. Interestingly, the results also show that arsenic content in clay reach about 6.00～36.00mg/kg,; 0.03～1.20mg/kg in pepper. The results suggest that arsenic contamination

also exists in the region. These results are presented in Table 1.

The characteristics of the study participants: The total number of participants was 196（Male 94 and female 102; 90% for farmers, 10% for workers). Their average age was 50.35±15.39 years; and body mass index was 22.34±2.78 kg/m^2. These results are presented in Table 2.

The influence of fluorine and arsenic on renal function

The effect of fluorine and arsenic on renal function: By observing the changes on renal function after exposure to different concentrations of fluoride and arsenic, it was shown that the content of UALB and UNAG in low fluoride group (p = 0.011, 0.000) and high fluoride group (p = 0.000, 0.000) are different compared to the normal fluoride group. Moreover, the content of Uα1-MG only in high fluoride group is different compared to the normal fluoride group (p = 0.000), but not in low fluoride group (p = 0.058). And the concentration of UALB, UNAG and Uα1-MG in low arsenic group (p = 0.000, 0.011, 0.000) and high arsenic group (p = 0.042, 0.004, 0.000) are different compared to the normal arsenic group. These results are presented in Table 3, 4.

The interaction between fluorine and arsenic interaction on renal function: Based on the concentration of fluoride and arsenic, UF below 1.2mg/gCr and UAs less than 20μg/gCr for the control group. It is a single exposure to arsenic if the group with normal UF and abnormal UAs, including As_{low} (UAs is between 20 to 40μg/gCr) and As_{high} (UAs is higher than 40μg/gCr) group. Meanwhile, the group with normal UAs and abnormal UF as the single exposure to fluoride, including F_{low} (UF is between 1.2 to 2.4mg/gCr) and F_{high} (UF is higher than 2.4mg/gCr) group. The interaction groups including $F_{low}As_{low}$ (UF is between 1.2 to 2.4mg/gCr and UAs is between 20 to 40μg/gCr) and $F_{low}As_{high}$ (UF is between 1.2 to 2.4mg/gCr and UAs is higher than 40μg /gCr) and $F_{high}As_{low}$ (UF is higher than 2.4mg/gCr and UAs is between 20 to 40μg/gCr) group. And the $F_{high}As_{high}$ (UF is higher than 2.4mg/gCr and UAs is higher than 40μg /gCr) group with no sample in this study. Compared with control group, UALB in As_{low} and F_{high} group (p = 0.016, 0.000) are different, but not in As_{high} and F_{low} group (p = 0.687, 0.238); UNAG in As_{high}, F_{low} and F_{high} group (p = 0.014, 0.024, 0.000) are different, but not in Aslow group (p = 0.575); Uα1-MG in As_{low}, As_{high}, F_{low} and F_{high} group (p = 0.003, 0.002, 0.024, 0.000) are different. UALB in $F_{low}As_{low}$, $F_{low}As_{high}$ and $F_{high}As_{low}$ group are higher than the single fluoride and arsenic exposure group (p = 0.000, 0.014; 0.000, 0.000; 0.000, 0.035). UNAG in $F_{low}As_{low}$, $F_{low}As_{high}$ and $F_{high}As_{low}$ group are

higher than the single fluoride and arsenic exposure group (p = 0.018, 0.080; 0.014, 0.002; 0.000, 0.042). Uα1-MG in $F_{low}As_{high}$ and $F_{high}As_{low}$ group are higher than the single fluoride and arsenic exposure group (p = 0.016, 0.003; 0.000, 0.011), but not in $F_{low}As_{low}$ group (p = 0.545, 0.062). These results were presented in Table 5. With covariates for regression models analysis, the results showed that single fluorine can affect the content of UALB, UNAG and Uα1-MG (*OR*=10.168, 7.864, 2.379; *95%CI*=6.045～17.104, 4.106～15.063, 1.436～3.942; p=0.000, 0.000, 0.001), single arsenic also can affect the content of UALB, UNAG and Uα1-MG (*OR*=3.714, 5.852, 2.907; *95%CI*=1.724～7.999, 2.331～14.695, 1.520～5.561; p=0.001, 0.000, 0.001). Meanwhile; the interaction analysis results showed that fluorine and arsenic can affect the content of UALB, UNAG and Uα1-MG (*OR*=0.285, 0.415, 0.037; *95%CI*=0.083～0.976, 0.032～0.654, 0.091～0.926; p=0.046, 0.012, 0.037). These results were presented in Table 6.

Risk assessment of fluoride and arsenic on renal function

The dose relationship assessment of fluorine and arsenic on renal function: The cutoff point was defined based on the 95% upper limit values, which were calculated from the control group. The cutoff values of UALB, UNAG and Uα1-MG were 145.63ng/gCr, 28.33ng/gCr, and 196.38ng/gCr. There are used to determine abnormal or normal. It was clearly shown that there were significantly increased at the abnormal rate of UALB, UNAG and Uα1-MG with increasing fluoride excretion ($\chi^2_{correlation}$ = 15.895, 15.633, 4.769; p = 0.000, 0.000, 0.029) and arsenic excretion ($\chi^2_{correlation}$ = 15.406, 5.702, 10.649; p = 0.000, 0.017, 0.001) in urine. These results are presented in Figure 1, 2.

The exposure assessment of fluorine and arsenic on renal function: Benchmark dose is calculated using the U.S. EPA BMDS version 2.4.0 software. The benchmark dose response was 5%, logistic model was chosen. It was demonstrated that the benchmark dose (BMD) and the 95% lower confidence limit of benchmark dose (BMDL) range co-exposed to fluoride and arsenic were determined; UF were 0.68 ~ 1.12mg/g Cr, 0.54 ~ 0.86mg/g Cr, and UAs were 13.06 ~ 23.30μg/g Cr, 10.50 ~ 17.40μg/g Cr. These results are shown in Table 7 and Figure 3, 4.

Discussion

Fluorine and arsenic are accumulation elements. In recent years, an increasing number of researchers are taking attention on the interaction between fluoride and arsenic. Although there

have been reports in literature of individual toxicity of arsenic and fluoride, however, there is very little known about the combined effects of these two elements, especially the impact on renal function in population. We observed the changes of renal function biomarkers after exposure to different concentrations of fluoride and arsenic, and to study the combined effects of fluoride and arsenic. In addition, benchmark dose method was used to explore exposure limits on renal function damage in population who co-exposure to fluoride and arsenic.

The results of the epidemiological investigation

In order to confirm the investigation area is a ward co-exposed to fluoride and arsenic, environmental samples were collected for the detection of fluoride and arsenic content. It was clear shown that the area have both fluoride and arsenic contamination. If people in the long-term exposure to this environment, they can intake of fluoride and arsenic by respiratory and digestive tract which can cause chronic poisoning.

The influence of fluorine and arsenic on renal function

Albumin is a normal protein in blood. Under normal circumstances, the large-sized proteins cannot pass through glomeruli, but when the kidneys are not working properly, it might pass through the glomeruli and find its way into the urine. It is an important indicator of glomerular and tubular damage. In the study, the results show that fluoride and arsenic play an important role in increasing the excretion of UALB. The content of UALB in $F_{low}As_{low}$, $F_{low}As_{high}$ and $F_{high}As_{low}$ group are higher than the group which single exposure to fluoride (F_{low} or F_{high} group) or arsenic (As_{low} or As_{high} group), but less than the sum of the two effects. Meanwhile, the covariate for regression models analysis is demonstrated that the change of UALB has a relationship with fluoride and arsenic. It showed that the change of UALB is the result of the combined effect of the fluorine and arsenic. There are have an 0.046 (coefficient) impact to the content of UALB (OR=0.285; 95%CI is 0.083～0.976) between fluoride and arsenic. These results suggest that fluoride and arsenic have an interaction on UALB, and there are mainly as antagonism in these exposure levels.

N-acety1-beta-D-glucosaminidase mainly comes from the renal proximal tubule epithelial cells, is an important lysosomal hydrolases. The content of this enzyme is very rich in the kidney tissue. Under normal circumstances, this enzyme cannot pass by glomerular filtration. Therefore, it is an important marker enzyme of tubular damage.[16] In the study, the results show that fluoride

and arsenic play an important role in increasing the excretion of UNAG. The content of UNAG in $F_{low}As_{low}$, $F_{low}As_{high}$ and $F_{high}As_{low}$ group are higher than the group which single exposure to fluoride (F_{low} or F_{high} group) or arsenic (As_{low} or As_{high} group), but less than the sum of the two effects. Simultaneously, the covariate for regression models analysis is demonstrated that the change of UNAG is the result of the combined effect of the fluorine and arsenic. There are have an 0.012 (coefficient) impact to the content of UNAG (OR=0.145; 95%CI is 0.032～0.654) between fluoride and arsenic. These results suggest that fluoride and arsenic have an interaction on UNAG, and there are mainly as antagonism in these exposure levels.

Alpha-1-micro-globulin is a micro globulin, a small globular protein.[17] It is found in all vertebrates, including humans, and is distributed in blood plasma and extravascular tissues of all organs. Uα1-MG can be used as an indicator of proteinuria. In the study, the results show that Uα1-MG change is the result of the impact of fluoride arsenic. The content of Uα1-MG in $F_{low}As_{high}$ and $F_{high}As_{low}$ group are higher than the group which single exposure to fluoride (F_{low} or F_{high} group) or arsenic (As_{low} or As_{high} group), but less than the sum of the two effects. There are no different between $F_{low}As_{low}$ group and single exposure to fluoride and arsenic group. At the same time, the covariate for regression models analysis is demonstrated that there are have an 0.037 (coefficient) impact to the content of Uα1-MG (OR=0.290; 95%CI is 0.091～0.926) between fluoride and arsenic. This result suggests that the effect between fluoride and arsenic on Uα1-MG as an independent action in the $F_{low}As_{low}$ group, but antagonism in the $F_{low}As_{high}$ and $F_{high}As_{low}$ group, for these exposure levels.

The above results suggests that the combined effect of fluoride and arsenic on renal function is mainly antagonism in this exposure levels. This is similar to the result of animal experiment that combined exposure to arsenic and fluoride does not necessarily lead to more pronounced toxicity and interestingly exhibit some antagonistic effects.[18] But in other study, children were exposed to arsenic and fluoride via drinking water, a higher level of apoptosis was detected in children with the highest exposure levels to both contaminants.[19] It have also found that combined fluoride and arsenic exposure did not have a more pronounced effect on spatial learning and memory compared with arsenic and fluoride exposure alone.[20] Moreover, in an experimental mouse model, tissue oxidative stress and cell injury were less pronounced in mice exposed to arsenic or fluoride compared to animals exposed to either arsenic or fluoride alone.[21] These studies suggest that

fluoride arsenic interaction is very complex, may be affected by a variety of factors, such as exposure dose, species, organ selectivity, and so on. Although the results are not consistent, it is very necessary to study on the joint action of these two toxicants. This study has some limitations such as: lack of a population with exposure to arsenic and fluoride alone, a small sample size. More studies are needed to clarify the associations shown by our results and to determine their effects on the renal function.

Risk assessment of fluorine and arsenic on renal function

Risk assessment is the systematic scientific evaluation of potential adverse health effects resulting from human exposures to hazardous agents or offices. The current risk assessment is mainly based on experimental data, exposure to fluoride and arsenic alone, and did not take into account the interaction between the fluoride and arsenic. Therefore, it is necessary to establish exposure limits on renal function damage in population who co-exposure to fluoride and arsenic.

Risk assessment is often used NOAEL method and the benchmark dose method. Advantages of the benchmark dose approach can include (1) the ability to take into account the dose-response curve; (2) the inclusion of a measure of variability (confidence limit); and (3) the use of a consistent benchmark response level for reference dose calculations across studies. Because of these advantages, the benchmark dose (BMD) approach was accepted by more and more people. The results indicate that the BMD and BMDL range co-exposed to fluoride and arsenic were determined, UF were 0.68 ~ 1.12mg/gCr, 0.54 ~ 0.86mg/gCr, and UAs were 13.06 ~ 23.30μg/gCr, 10.50 ~ 17.40μg/gCr. From a security point of consideration, the critical concentrations of UF and UAs for renal function were estimated as 0.54mg/g creatinine and 10.50μg/g creatinine in population co-exposed to fluoride and arsenic, respectively.

In the study, the critical concentrations of UF and UAs were lower than the ACGIH biological exposure index (the critical concentrations of UF is 2 μg/mL, equivalent 1.2mg/gCr; UAs is 35μg/l, equivalent 20μg/gCr;). What causes these changes? Well I think there are two reasons. In the first place, the interventions (Improving stove and health education) lead to fluoride and arsenic exposure reduced, and the excretion of fluoride and arsenic reduced too. Secondly, the current standard is mainly based on observational data, exposure to fluoride and arsenic alone, but this critical concentration calculated by co-exposure to fluoride and arsenic. In addition, When UALB as effect biomarker, the benchmark dose value was lower than UNAG and Uα1-MG. These

results show the sensitivity of UALB is higher than UNAG and Uα1-MG in the evaluation of renal function damage caused by fluoride and arsenic. It indicates that UALB can be used as a relative sensitive biomarker of renal function in population who co-exposed to fluoride and arsenic.

Conclusions

In summary, we have concluded that fluoride and arsenic can induced the glomerular and tubular damage. In this exposure levels, the combined effect of fluoride and arsenic on renal function is mainly antagonism. Moreover, the critical concentrations of UF and UAs for renal function damage were estimated as 0.54mg/gCr and 10.50μg/gCr in population co-exposed to fluoride and arsenic, respectively; and UALB can be used as a relative sensitive biomarker of renal function.

Conflict of interest statement

Nothing to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 30660163, 81072248) and the youth fund of Guiyang Medical University (NO. 2012027, 2012028).

References

1. Chouhan S, Flora SJ. Arsenic and fluoride: two major ground water pollutants. Indian journal of experimental biology. 2010;48:666-678.
2. Jia L, Jin TY. Combined effect of fluoride and arsenate on gene expression of osteoclast differentiation factor and osteoprotegerin. Biomedical and environmental sciences : BES. 2006;19:375-379.
3. Zeng QB, Xu YY, Yu X, Yang J, Hong F, Zhang AH. Arsenic may be involved in fluoride-induced bone toxicity through PTH/PKA/AP1 signaling pathway. Environmental toxicology and pharmacology. 2014;37:228-233.
4. Yamauchi H, Yamamura Y. Metabolism and excretion of orally administrated arsenic trioxide in the hamster. Toxicology. 1985;34:113-121.
5. Marafante E, Bertolero F, Edel J, Pietra R, Sabbioni E. Intracellular interaction and biotransformation of arsenite in rats and rabbits. The Science of the total environment. 1982;24:27-39.
6. Robles-Osorio ML, Perez-Maldonado IN, Martin del Campo D, et al. Urinary arsenic levels and risk of renal injury in a cross-sectional study in open population. Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion. 2012;64:609-614.
7. Lee JY, Eom M, Yang JW, Han BG, Choi SO, Kim JS. Acute kidney injury by arsine poisoning: the ultrastructural pathology of the kidney. Renal failure. 2013;35:299-301.
8. Feng H, Gao Y, Zhao L, et al. Biomarkers of renal toxicity caused by exposure to arsenic in drinking water. Environmental toxicology and pharmacology. 2013;35:495-501.
9. Hamilton IR. Biochemical effects of fluoride on oral bacteria. Journal of dental research. 1990;69 Spec No:660-667; discussion 682-663.
10. Kaminsky LS, Mahoney MC, Leach J, Melius J, Miller MJ. Fluoride: benefits and risks of exposure. Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists. 1990;1:261-281.
11. Santoyo-Sanchez MP, del Carmen Silva-Lucero M, Arreola-Mendoza L, Barbier OC. Effects of acute sodium fluoride exposure on kidney function, water homeostasis, and renal handling of calcium and inorganic phosphate. Biological trace element research. 2013;152:367-372.
12. Yang SY, Zhang L, Miao KK, Qian W, Zhang ZG. Effects of selenium intervention on chronic

fluorosis-induced renal cell apoptosis in rats. Biological trace element research. 2013;153:237-242.

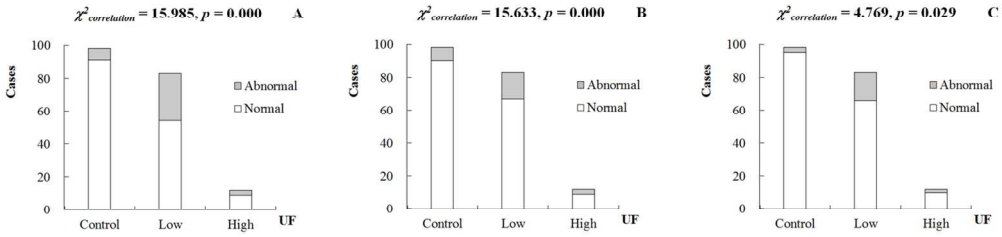
13. An D, Li D, Liang Y, Jing Z. Unventilated indoor coal-fired stoves in Guizhou province, China: reduction of arsenic exposure through behavior changes resulting from mitigation and health education in populations with arsenicosis. Environ Health Perspect. 2007 Apr;115(4):659-62.
14. Zhang A, Feng H, Yang G, Pan X, Jiang X, Huang X, Dong X, Yang D, Xie Y, Peng L, Jun L, Hu C, Jian L, Wang X. Unventilated indoor coal-fired stoves in Guizhou province, China: cellular and genetic damage in villagers exposed to arsenic in food and air. Environ Health Perspect. 2007 Apr;115(4):653-8.
15. An D. The prevention and practice of coal burning endemic fluorosis. Guiyang: Guizhou Science and Technology Press, China. 2011; 1.
16. Idasiak-Piechocka I, Krzymanski M. [The role of tubulointerstitial changes in progression of kidney function failure in patients with chronic glomerulonephritis (GN)]. Przeglad lekarski. 1996;53:443-453.
17. Ekstrom B, Peterson PA, Berggard I. A urinary and plasma alpha1-glycoprotein of low molecular weight: isolation and some properties. Biochemical and biophysical research communications. 1975;65:1427-1433.
18. Flora SJ, Mittal M, Mishra D. Co-exposure to arsenic and fluoride on oxidative stress, glutathione linked enzymes, biogenic amines and DNA damage in mouse brain. J Neurol Sci. 2009 Oct 15;285(1-2):198-205.
19. Rocha-Amador DO, Calderón J, Carrizales L, Costilla-Salazar R, Pérez-Maldonado IN. Apoptosis of peripheral blood mononuclear cells in children exposed to arsenic and fluoride. Environmental toxicology and pharmacology. 2011 Nov;32(3):399-405.
20. Jiang S, Su J, Yao S, Zhang Y, Cao F, Wang F, Wang H, Li J, Xi S. Fluoride and Arsenic Exposure Impairs Learning and Memory and Decreases mGluR5 Expression in the Hippocampus and Cortex in Rats. PLoS One. 2014 Apr 23;9(4):e96041.
21. Mittal M, Flora SJ. Vitamin E supplementation protects oxidative stress during arsenic and fluoride antagonism in male mice. Drug and chemical toxicology. 2007; 30:263-281.

Fig.1 The dose relationship between UF and UALB (A), UNAG (B) and Uα1-MG (C). It was clearly shown that there were significantly increased at the abnormal rate of UALB, UNAG and Uα1-MG with increasing fluorine excretion in urine ($p<0.05$ or $p<0.01$).

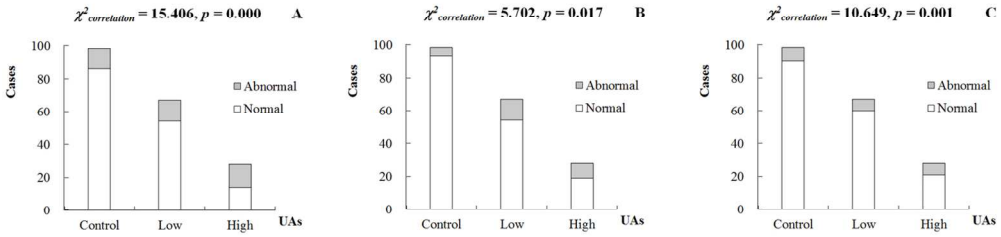
Fig.2 The dose relationship between UAs and UALB (A), UNAG (B) and Uα1-MG (C). It was clearly shown that there were significantly increased at the abnormal rate of UALB, UNAG and Uα1-MG with increasing arsenic excretion in urine ($p<0.05$ or $p<0.01$).

Fig.3 With UALB (A), UNAG (B) and Uα1-MG (C) as effect biomarkers, the BMD and BMDL curve of UF. It was clearly shown that the BMD and BMDL range of UF 0.68 ~ 1.12mg/g Cr, 0.54 ~ 0.86mg/g Cr.

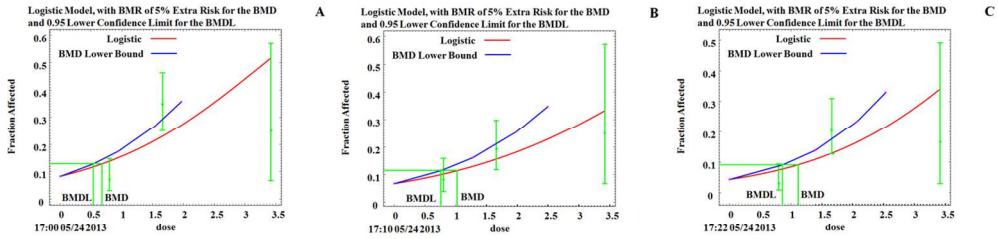
Fig.4 With UALB (A), UNAG (B) and Uα1-MG (C) as effect biomarkers, the BMD and BMDL curve of UAs. It was clearly shown that the BMD and BMDL range of UAs were 13.06 ~ 23.30μg/g Cr, 10.50 ~ 17.40μg/g Cr.



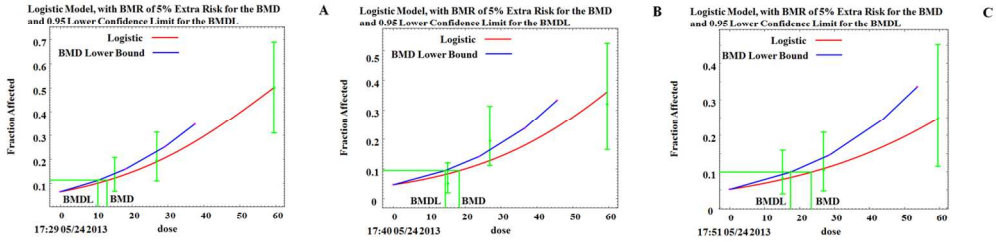
130x50mm (299 x 299 DPI)



130x50mm (299 x 299 DPI)



130x50mm (299 x 299 DPI)



130x50mm (299 x 299 DPI)