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Confounding Effects of Primary Hepatocellular Carcinoma on Urinary Arsenic as an Environmental Exposure Indicator in a Karst High-Background Population

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Abstract

Arsenic (As) concentrations in bodily fluids are widely used to assess external environmental exposure, governed jointly by external intake and internal metabolism. However, current research largely overlooks the confounding effects of primary hepatocellular carcinoma (PHC) on these biomarkers. To clarify the utility of bodily fluid As as an environmental exposure indicator, we first conducted an exposure association study across five villages in Guangxi, China. Total As levels in water, soil, and rice were measured to estimate multi-pathway external exposure doses, while blood and urine samples from long-term residents were synchronously collected and analyzed to elucidate the relationship between external exposure and internal As burden. Subsequently, a comparative study between a healthy population and PHC patients was conducted to evaluate the interference of PHC on fluid As distribution. Results indicated that rice consumption constituted the primary exposure pathway, contributing 66.32% to 95.25% of the total intake. In the healthy group, the geometric mean of urinary As (33.96 μg/L) was significantly higher than that of blood As (2.02 μg/L). Notably, in the PHC group, both urinary As (4.55 μg/L) and blood As (1.81 μg/L) were lower than those in the Normal group. Receiver Operating Characteristic (ROC) curve analysis confirmed that urinary As was highly confounded by PHC (Area Under the Curve [AUC] = 0.916). In conclusion, threshold evaluations based solely on environmental media fail to accurately reflect the true internal As burden. While urinary and blood As are generally effective indicators of human exposure, the onset of PHC induces an abnormal decline in urinary As. Consequently, relying on urinary As may lead to a critical

underestimation of environmental As exposure risks in PHC populations.

Keywords: Arsenic exposure; Primary hepatocellular carcinoma; Biomonitoring; Biomarkers; Risk assessment

Introduction

Arsenic (As) is classified as a Group I human carcinogen [1], and accurately assessing multi-pathway As exposure in populations residing in regions with high geological backgrounds remains a formidable challenge. Traditional environmental risk assessments primarily estimate external exposure doses based on As concentrations in environmental media. However, discrepancies frequently occur between these estimated external doses and actual internal As accumulation, making it difficult to accurately determine the health risks of populations in high-As endemic areas [2-5]. Consequently, total blood As and urinary As have been widely adopted as biomarkers of internal exposure in epidemiological studies to reflect the true internal As burden [6-8]. However, As concentrations in blood and urine are governed by the dual mechanisms of external environmental exposure and internal physiological metabolism [9-10]. Current environmental epidemiological studies predominantly focus on the causal association between As exposure and primary hepatocellular carcinoma (PHC) [11-12]. Yet, they largely overlook whether severe pathological damage induced by carcinogenesis in the liver—the central hub of As metabolism—alters the distribution patterns of As in bodily fluids. While existing evidence confirms that liver cirrhosis can alter the proportions of urinary As species [9,13], these findings cannot be directly extrapolated to populations with primary cancer. This gives rise to a critical paradox: in field settings characterized by high geological backgrounds and complex exposures, biomarkers inherently intended to indicate external environmental exposure are highly susceptible to severe confounding by the pathological states of diseased cohorts. This, in turn, can lead to gross misestimations of exposure levels or misjudgments of health risks.

Therefore, elucidating the confounding effects of PHC inherently requires establishing a reliable metabolic baseline in a healthy population. However, under multi-pathway complex exposure scenarios involving water, soil, and rice, there remains a glaring paucity of quantitative baseline studies addressing the dose-response relationship between external exposure and bodily fluid As in healthy cohorts [14-16]. It remains inconclusive whether individual variations in bodily fluid As concentrations, even under identical external exposure doses, stem from differences in the proportional weights of multi-media exposure routes or from individual metabolic dysregulation [17-19]. Directly applying evaluation criteria derived from single-source exposure models to populations with a high incidence of liver disease will undoubtedly and severely compromise the reliability of health risk assessments in high-background regions.

To address these scientific gaps, this study focuses on a typical high-As karst geological background region in Guangxi, China. By conducting a cross-sectional comparative study that integrates an environmental population cohort with a clinical cohort, we aim to transcend the inherent limitations of traditional single-biomarker evaluations. Our specific objectives are: first, to reveal the response patterns and baseline characteristics of blood and urinary As to exogenous inputs among healthy populations under multi-media complex exposures; second, to quantify the confounding impact of PHC on blood and urinary As levels; and third, to evaluate the practical utility of blood and urinary As for assessing As exposure in regions with a high prevalence of liver disease. By elucidating the dual-driving mechanisms of environmental inputs and hepatic pathology on these biomarkers, this study not only provides a more precise framework for exposure assessment in areas where high geological backgrounds overlap with underlying endemic diseases, but also significantly enriches the theoretical basis of internal exposure evaluation in environmental epidemiology.

Materials and Methods

Study area and participant recruitment

This study was conducted in a typical high geological background karst region in Guangxi, China. Based on preliminary regional geochemical surveys, soil element background data, and micro-geomorphological characteristics, five villages (Celing, Gula, Qingfeng, Xingui, and Zhenliang) were selected as environmental investigation areas. Selection criteria explicitly excluded known mining areas and industrial point-source pollution sites to avoid anthropogenic confounding.

This study was conducted in strict accordance with the ethical principles of the Declaration of Helsinki. Human blood and urine data were derived from the Guangxi Key Research and Development Project, " Multicenter Study on Carcinogenic Effects and Spatial Prediction Model of Heavy Metal Exposure in High-Incidence Areas of Primary Liver Cancer in Guangxi " (Grant No. AB23026041). The study protocol was reviewed and approved by the Medical Ethics Committee of Guangxi Medical University (Approval No.: 2022–0181). All participants provided written informed consent prior to enrollment.

Three distinct population cohorts were included in this study: (1) Environmental cohort: 267 long-term residents with no history of occupational exposure were screened and recruited from the five high-As karst background villages. Blood and urine samples were collected, alongside evenly distributed local soil and rice samples, and surface water As data were compiled. (2) Healthy control group: 105 healthy individuals, confirmed to be free of hepatic and renal diseases by the hospital's medical examination department. (3) Diagnosed PHC case group: 105

patients clinically diagnosed with primary hepatocellular carcinoma (PHC) at the hospital.

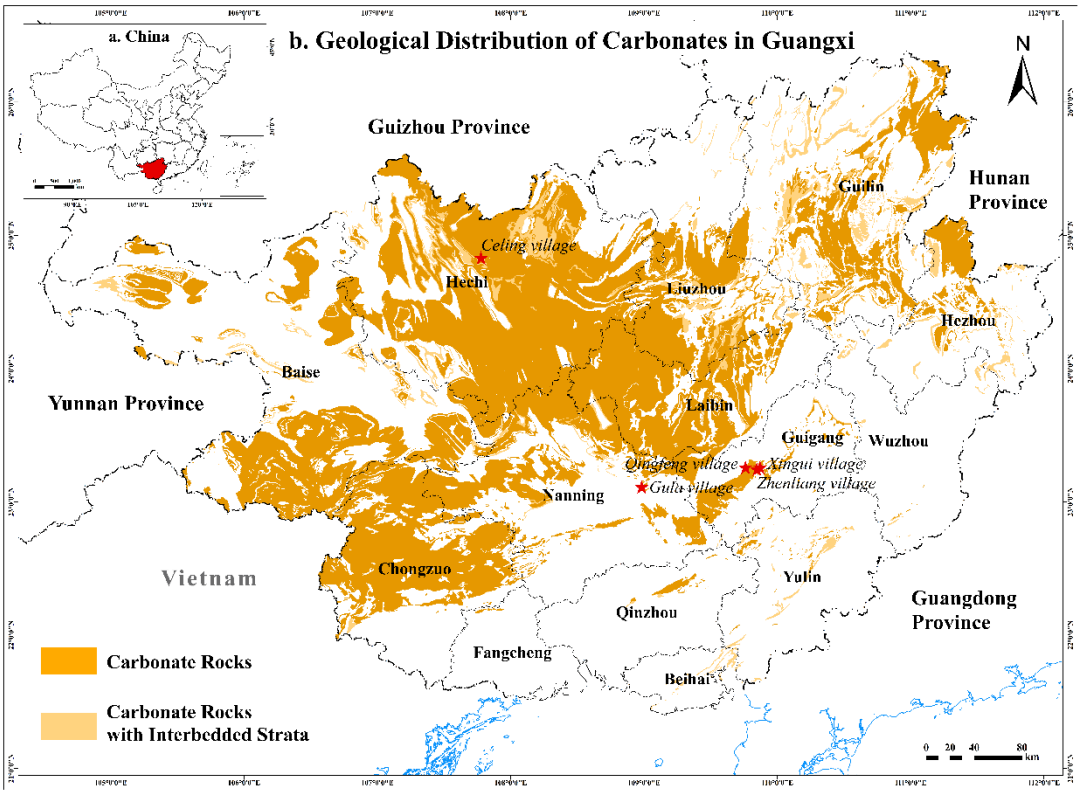


Figure 1. Spatial distribution of the typical study areas.

The area falls in a subtropical monsoon climate zone, having a mean annual temperature of 19.1°C. The long-term average annual precipitation is 1,596 mm, as per records from 1961 to 2003, with August being the peak rainfall month, during which the average monthly precipitation exceeds 300 mm. The region is primarily underlain by sedimentary rock strata, including the Cambrian, Devonian, Carboniferous, and Cretaceous systems. Its lithology is dominated by carbonate rocks, while some clastic rocks are also present, mainly occurring as interlayers or alternating layers. The major fault zones in the area trend NW and NE, and have undergone multiple episodes of tectonic reactivation. These faults are characterized by widely exposed fracture zones, and well-developed joint systems are commonly observed in their vicinity.

Sample collection

For soil and rice samples, to ensure precise matching with individual dietary exposure, conventional grid sampling was not adopted. Instead, staple food sources were traced via questionnaires, and paired samples of mature rice grains and corresponding rhizosphere soil (0–20 cm depth) were collected directly from the residents' own farmlands (N = 129 pairs). Soil samples were naturally air-dried, ground, and passed through a 10-mesh nylon sieve. Rice grains were de-husked, oven-dried, and pulverized for subsequent analysis.

Fasting venous blood samples (5 mL) were collected in the morning by professional medical staff using vacuum tubes containing EDTA-K2 anticoagulant. Samples were gently inverted to mix, transported to the laboratory via a cold chain within 8 hours, and stored at -80°C in an ultra-low temperature freezer. For urine samples, 10 mL of midstream morning urine was collected from each participant, aliquoted into sterile centrifuge tubes, and cryopreserved at -80°C. Urinary As concentrations were subsequently measured as total As.

Notably, although residents in the five villages currently rely on centralized tap water as their primary drinking source, this study aimed to explore the macro-ecological association between the regional high-As background and internal exposure, rather than achieving precise individual-level household exposure matching. Therefore, environmental water As exposure levels were spatially extracted from the surface water database of the Guangxi Land Quality Geochemical Dataset using Geographic Information Systems (GIS), covering 29 surface water monitoring sites across the targeted villages and surrounding major river networks. These environmental surface water As concentrations macroscopically and authentically reflect the overall As exposure pressure via drinking water, serving as the ecological baseline parameter for calculating the Estimated Daily Intake of water (EDIwater) for the regional population.

Chemical analysis and quality control

All heavy metal analyses were conducted in certified laboratories. Whole blood and urine samples were pre-treated via microwave digestion using an HNO_3-H_2O_2 system. After centrifugation at 2000 r/min for 2 min, the supernatant was collected, and total As concentrations in blood and urine were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, G2000).

Rice grains were washed with pure water, oven-dried at a low temperature, de-husked, rinsed with deionized water, and dried again in a 55°C oven. The samples were pulverized to a uniform size of 40–60 mesh and sieved. Exactly 1 g of the processed sample was weighed and subjected to hot extraction with 20 mL of dilute nitric acid (0.15 mol/L) at 90°C for 2.5 h. After cooling to room temperature, As concentrations were determined via Liquid Chromatography-Atomic Fluorescence Spectrometry (LC-AFS).

Soil samples were oven-dried and pulverized to 200 mesh. A 0.2 g aliquot of the sample was digested in a boiling water bath with a (1+1) aqua regia solution and diluted to 25 mL with water. A thiourea-ascorbic acid mixture was added as a reducing agent, and potassium borohydride was used as a hydride generator. As concentrations were then determined using Atomic Fluorescence Spectrometry (AFS).

The analytical process rigorously incorporated blank controls, duplicate samples, and standard reference material (SRM) recovery tests for quality control. Soil (GBW7425/GSS-11, etc.), rice (GBW(E)100348, etc.), blood

(Seronorm Trace Elements Blood L-1/L-2, Lot 2011934), and urine SRMs (Seronorm Trace Elements Urine L-1/L-2, Lot 1706877) were utilized to monitor instrumental accuracy. The limits of detection (LOD) for As in water, bodily fluids, soil, and rice were <0.13 μg/L, <0.13 μg/L, <0.2 mg/kg, and <0.02 mg/kg, respectively. SRM recovery rates were maintained between 90% and 110%, and the relative standard deviation (RSD) of duplicate samples was <5%. For measurements below the LOD, a value of LOD/2 was substituted for subsequent statistical analyses.

Exposure assessment

To quantify the transfer of As from environmental media to the human body in high-background regions, human health risk assessment and heavy metal intake evaluation models were applied. The Estimated Daily Intake (EDI) [mg/(kg·d)] via multiple pathways was calculated using the following formulas:

$$EDI_{\text{water}} = \frac{C_{water} \times IR}{BW} \quad (1)$$

$$EDI_{\text{rice}} = \frac{C_{rice} \times IR}{BW} \quad (2)$$

$$EDI_{\text{soil}} = \frac{C_{soil} \times IR \times CF}{BW} \quad (3)$$

$$EDI_{\text{total}} = EDI_{\text{soil}} + EDI_{\text{rice}} + EDI_{\text{water}} \quad (4)$$

Where C_{water} , C_{rice} and C_{soil} represent the total As concentrations in surface water (μg/L), rice (mg/kg), and soil (mg/kg), respectively. IR_{water}, IR_{rice} and IR_{soil} denote the ingestion rates of water (L/d), rice (g/d), and soil (mg/d), respectively. BW represents the average body weight (kg), and CF refers to the unit conversion factors (10^{-3} and 10^{-6} as applicable).

Based on local parameters and previous literature [19-21], the exposure parameters adopted in this study were: daily rice intake of 238.3 g/d, daily water intake of 1.85 L/d, daily soil intake of 100 mg/d, and an average body weight of 60.6 kg.

Statistical analysis

Statistical analyses were performed using SPSS 26.0, and data visualizations were generated using R 4.2.1 and MapGIS. The Shapiro–Wilk test was employed to evaluate the distribution characteristics of continuous variables. Because total As concentrations in environmental and biological samples typically exhibit skewed distributions, continuous variables were described using both arithmetic and geometric means. To eliminate the confounding

effects of age, gender, and individual variations on overall trends, the environmental cohort assessment was conducted using the village as the statistical unit. Stacked bar-line charts were utilized to conduct trend analyses on the relationships between environmental exposure indicators and total As in blood and urine.

In the clinical case-control module, Receiver Operating Characteristic (ROC) curves were initially employed to evaluate the independent discriminatory ability of total urinary As and blood As in distinguishing PHC patients from Normal group. Given the differences in age and gender distributions between the two clinical cohorts, a multivariable binary logistic regression model was further constructed, incorporating age and gender as covariates for statistical adjustment. Subsequently, the predicted probabilities generated by this regression model were extracted as new test variables to plot an Adjusted ROC curve, thereby eliminating the confounding interference of demographic characteristics on the evaluation of diagnostic efficacy. The overall discriminatory performance of the models was quantified using the Area Under the Curve (AUC) and its 95% confidence interval (CI), with the optimal cut-off value determined by maximizing Youden's index. All statistical tests were two-tailed, and a P-value < 0.05 was considered statistically significant.

Results

As concentrations in environmental media and exposure burden

Table 1 summarizes the As concentrations and sample exceedance rates across three environmental media (surface water, rice, and soil) in the five studied villages, alongside the estimated multi-pathway As exposure doses for the local populations. As concentrations in the environmental media exhibited distinct spatial heterogeneity. Overall, As pollution in the study area was characterized by widespread exceedance in rice, accompanied by localized exceedances in water and soil. Surface water As pollution was primarily concentrated in Celing and Xingui villages, with mean concentrations of 14.15 ± 10.03 μg/L and 9.30 ± 4.89 μg/L, respectively. Based on the total As evaluation limit of 10 μg/L (0.01 mg/L) [22], both villages recorded an exceedance rate of 50%. In contrast, surface water As concentrations in the remaining villages were maintained at low levels, with no samples exceeding the standard. Regarding rice, the mean total As concentrations across the five villages ranged from 0.22 to 0.29 mg/kg. Using the inorganic As limit of 0.20 mg/kg for rice as a reference threshold [23], the exceedance rates across the five villages ranged from 69.05% to 100%, with Gula village notably exhibiting a 100% exceedance rate. For soil, the mean As concentrations in Qingfeng, Xingui, and Zhenliang villages were 45.89, 71.86, and 49.49 mg/kg, respectively. These values surpassed the soil risk screening value of 30 mg/kg [24], resulting in exceedance rates of 80.95%, 65.00%, and 38.46%, respectively. Conversely, Celing village had zero

soil exceedances, and Gula village recorded an exceedance rate of only 8.33%, indicating pronounced inter-village variations in soil As backgrounds.

Multi-pathway exposure assessments revealed that the total Estimated Daily Intake (EDI) across the five villages ranged from 1.02 to 1.42 μg/(kg·d). While the total exposure levels were comparable among the villages, the contributions of specific exposure pathways varied significantly, with rice consumption emerging as the predominant exposure route. The EDIrice across the villages was maintained between 0.86 and 1.15 μg/(kg·d), accounting for 66.32% to 95.25% of the total exposure, thus constituting the most critical exposure source. Particularly in Gula village, the exposure contribution from rice reached 95.25%, indicating that human As exposure in this area was almost entirely driven by dietary intake. Spatial variations in EDIwater aligned with the surface water pollution profiles. Celing and Xingui villages exhibited relatively higher EDIwater, with drinking water exposure contributing 33.29% and 20.36%, respectively, rendering it a secondary exposure pathway in these areas. In all other villages, the drinking water contribution remained below 10%. Furthermore, despite exceptionally high and severely non-compliant soil As concentrations in certain villages, the EDI via incidental soil ingestion was consistently negligible. The contribution rate of soil exposure was below 8% across all villages, implying a limited overall impact on total human As exposure. In summary, although the total EDI (1.02–1.42 μg/[kg·d]) indicated comparable overall exposure burdens across the five villages, the compositional structure of the exposure pathways exhibited pronounced spatial divergence.

Table 1. Total arsenic concentrations in environmental media and estimated daily intake across five study villages.

Indicators		*Celing*	*Gula*	*Qingfeng*	*Xingui*	*Zhenliang*
A. Environmental As Concentrations	Local water, μg/L	14.15±10.03	0.83±0.85	2.66±2.02	9.3±4.89	1.88±1.06
	Rice, mg/kg	0.23±0.05	0.29±0.06	0.22±0.04	0.26±0.04	0.25±0.04
	Soil, mg/kg	3.12±1.63	18.28±13.10	45.89±24.10	71.86±62.18	49.49±58.35
	Water(Exceeding rate)	50%	0%	0%	50%	0%
	Rice(Exceeding rate)	73.33%	100.00%	69.05%	85.00%	92.31%
	Soil(Exceeding rate)	0.00%	8.33%	80.95%	65.00%	38.46%
	Water(N)	2	5	13	2	7
	Rice(N)	30	24	42	20	13
	Soil(N)	30	24	42	20	13
B. EstimatedDaily Intake,EDI,μg/kg bw/day	*EDIwater*	0.43	0.03	0.08	0.28	0.06
	EDIrice	0.89	1.15	0.86	1.02	0.99
	EDIsoil	0.01	0.03	0.08	0.12	0.08
	TotalEDI	1.33	1.20	1.02	1.42	1.13
C.Contribution Rate,%	*CRwater*,%	33.29%	2.15%	8.12%	20.36%	5.18%
	CRrice,%	66.32%	95.25%	84.28%	71.76%	87.65%
	CRsoil,%	0.39%	2.60%	7.60%	7.88%	7.16%

Note: As, arsenic; N, number of samples; SD, standard deviation; EDI, estimated daily intake; bw, body weight; CR, contribution rate. (A) Environmental As concentrations are presented as Mean ± SD. (B) EDIs for Local water,

208 rice, and soil were calculated using a deterministic point-estimate approach based on the mean As concentrations
209 of the respective environmental media. Total EDI is the sum of EDIwater, EDIrice, and EDIsoil. (C) Contribution
210 rate (CR) represents the percentage proportion of each specific exposure pathway relative to the Total EDI.

211 ***Impact of external exposure on bodily fluid As biomarkers***

212 Building upon the structural characteristics of multi-pathway As exposure in the study area, we further analyzed
213 the internal distribution profiles of As across different populations. Table 2 summarizes the total As concentrations
214 in the blood and urine of the healthy control group, the PHC case group, and the residents from the five surveyed
215 villages. Total blood As concentrations among the residents of the five villages remained generally stable, with
216 geometric means (GMs) ranging from 0.99 to 1.47 μg/L, indicating minor inter-village variations. Notably, Gula
217 village, which had the highest exposure contribution from rice, also exhibited the highest blood As level; this
218 finding aligns consistently with the baseline accumulation pattern of long-term dietary exposure.
219 In contrast, the inter-village variations in total urinary As were substantially more pronounced than those in blood
220 As, with GMs ranging widely from 8.58 to 36.16 μg/L. Furthermore, urinary As levels did not exhibit a monotonic
221 trend with the total EDI. Specifically, Celing and Xingui villages, which had relatively higher contributions from
222 drinking water, displayed elevated urinary As levels. Conversely, Gula village, where rice exposure was absolutely
223 dominant, recorded the lowest urinary As concentration. A quintessential example is Gula village: although the
224 exposure contribution from rice was as high as 95.25%, the GM of urinary As was merely 8.58 μg/L. In stark
225 contrast, in Celing village, where drinking water exposure accounted for 33.29% of the total intake, the GM of
226 urinary As surged significantly to 36.16 μg/L.
227 According to the trial standards issued by the Ministry of Health of China, the recommended guiding limit for the
228 population GM of urinary As is 32 μg/L [25]. Among the surveyed environmental cohorts, only the population in
229 Celing village exceeded this guiding limit, while the GMs in the other four villages remained below the threshold.
230 It is noteworthy that the urinary As GM of the healthy control group (33.96 μg/L) was also slightly above this
231 guiding limit. This suggests that even in regions without high-As drinking water, background dietary As exposure
232 can still drive population urinary As levels close to, or slightly beyond, this regulatory threshold.
233 Table 2. Total arsenic concentrations in blood and urine among Normal group, PHC patients, and community
234 participants from five villages.

Variables		*male* *n*（%）	*Age (yr)* *(Mean±SD)*	*Blood As, μg/L*		*Urine As, μg/L*	
				Mean±SE	Geometric Median	Mean±SE	Geometric Median
A. Clinical Cohort	Normal	male40（38%）	38.05±10.37	2.38±0.26	2.02	43.06±4.27	36.82

	Controls	female65（62%）	36.43±8.65	2.88±0.44	2.03	44.36±4.77	32.32
		total105	37.05±9.33	2.69±0.29	2.02	43.86±3.36	33.96
	PHC Patients	Male80（77%）	58.14±9.69	2.63±0.27	1.95	10.35±1.58	4.93
		Female 25（23%）	63.04±13.00	1.79±0.27	1.43	7.50±2.73	3.49
		105	59.28±10.69	2.43±0.22	1.81	9.68±1.37	4.55
B.Environmental Cohort	Celing	male 17（34%）	70.88±4.12	1.13±0.08	1.09	46.17±7.09	37.76
		female 33（66%）	73.82±6.96	0.99±0.05	0.95	40.42±3.87	35.36
		total50	72.82±6.26	1.04 ± 0.04	0.99	42.37 ± 3.49	36.16
	Gula	male 12（31%）	70.33±6.02	1.71±0.15	1.63	13.05±4.74	7.11
		female 27（69%）	73.44±6.71	1.52±0.13	1.4	14.45±2.71	9.33
		total 39	72.49±6.59	1.58 ± 0.10	1.47	14.02 ± 2.35	8.58
	Qingfeng	male 16（32%）	70.50± 5.99	1.09± 0.11	1.02	24.28± 3.77	20.63
		female 34（68%）	70.88± 8.12	1.19± 0.20	0.98	34.75± 4.18	26.95
		total 50	70.76±7.44	1.16 ± 0.14	0.99	31.40 ± 3.15	24.74
	Xingui	male 15（35%）	70.53±3.98	1.51±0.21	1.38	27.44±5.03	22.38
		female 28（65%）	71.86±3.85	1.19±0.07	1.15	22.70±3.12	16.64
		total43	71.40±3.90	1.30 ± 0.09	1.22	24.36 ± 2.67	18.46
	Zhenliang	male 33（39%）	74.27±5.78	1.33±0.10	1.24	33.25±3.81	22.51
		female 52（61%）	72.13±7.28	1.05±0.06	0.98	48.01±6.68	32.89
		total85	72.96±6.78	1.16 ± 0.05	1.08	42.28 ± 4.40	28.39

Note: Total As concentrations were determined in both blood and urine samples. Urinary As levels are reported in μg/L, and no adjustments for urinary creatinine or specific gravity were applied.

By superimposing the multi-pathway EDI onto the internal bodily fluid As burden (Figure 2), it becomes evident that the two biomarkers exhibit distinctly different response patterns. Despite the comparable total EDIs across the five villages, blood As consistently maintained a narrow range of fluctuation, thereby stably reflecting the long-term exposure baseline dominated by rice consumption. In contrast, urinary As exhibited dramatic fluctuations, with its elevated levels aligning closely with the increased exposure contributions from the drinking water pathway (as observed in Celing and Xingui villages).

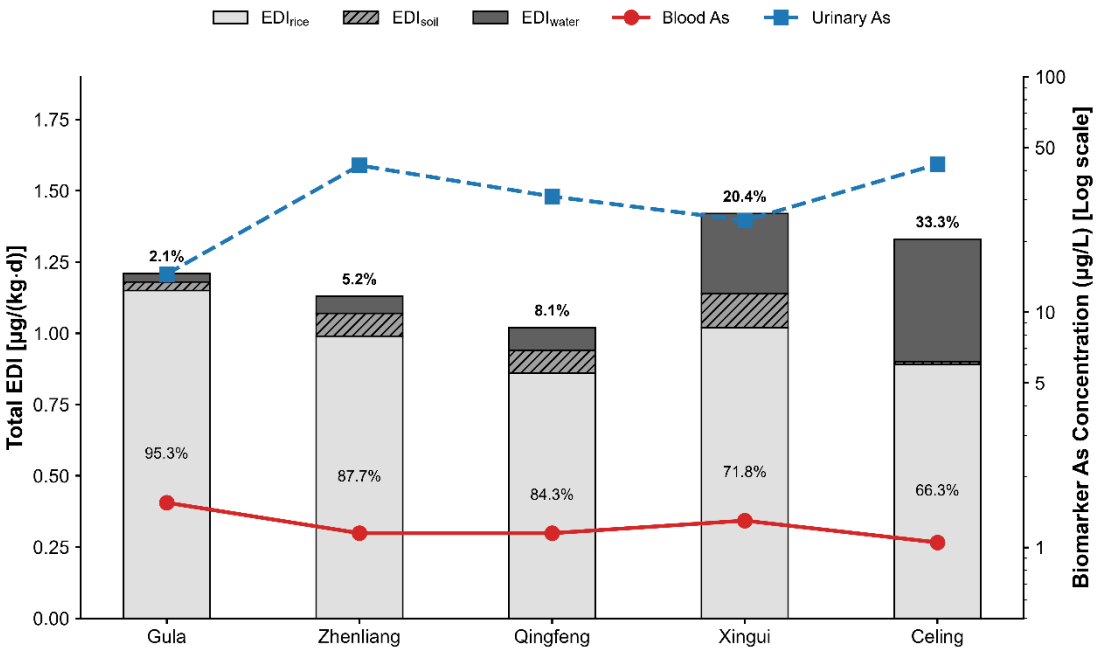


Figure 2. Integration of multi-pathway arsenic exposure (EDI) and corresponding human internal burdens across five studied villages.

NOTE:The stacked bar charts (left y-axis) delineate the absolute Estimated Daily Intake [EDI, μg/(kg·d)] derived from rice, soil, and water ingestion. Superimposed lines (right y-axis, plotted on a logarithmic scale) illustrate the biological monitoring results: arsenic concentrations (μg/L) in human blood (solid red line) and urine (dashed blue line). Percentages embedded within and above the bars denote the relative contribution rates of rice and drinking water to the overall environmental exposure, respectively. Notably, while rice remains the dominant exposure pathway globally, urinary arsenic levels exhibit pronounced fluctuations that align with the shifting exposure profiles (e.g., significantly increased water contributions in Xingui and Celing).

Characteristics of bodily fluid As in the PHC and healthy control groups

Inter-group comparisons within the clinical cohorts revealed statistically significant differences in the distribution of bodily fluid As between Normal group and PHC patients (Table 2A). In the healthy control group, the geometric mean (GM) of total blood As was 2.02 μg/L, whereas the GM of total urinary As reached 33.96 μg/L. This exhibits a typical distribution profile where urinary As concentrations are substantially higher than those in blood. Conversely, in the PHC case group, total blood As exhibited only a marginal decrease, with a GM of 1.81 μg/L; however, total urinary As underwent a precipitous decline, plummeting to a GM of merely 4.55 μg/L. This trend remained consistently robust following gender stratification. Within the PHC group, the urinary As GMs for males and females were 4.93 μg/L and 3.49 μg/L, respectively, both of which were significantly lower than those of their healthy counterparts (36.82 μg/L for healthy males and 32.32 μg/L for healthy females).

Spearman correlation analysis (Figure 3) demonstrated a significant positive correlation between blood As and urinary As in both the healthy control and PHC groups. The correlation coefficient (rsrs) was 0.469 (P<0.001) for the normal control group and 0.414 (P<0.001) for the PHC group. This indicates an inherent association between blood and urinary As across both populations, suggesting that the intrinsic metabolic coupling between blood and urinary As is not completely severed in patients with liver cancer. Nevertheless, as visualized in the distribution profiles (Figure 4), the overall distribution range of bodily fluid As in PHC patients exhibited a marked shift compared to the normal cohort. Ultimately, the PHC population manifested a distinctively "low urinary As" distribution characteristic.

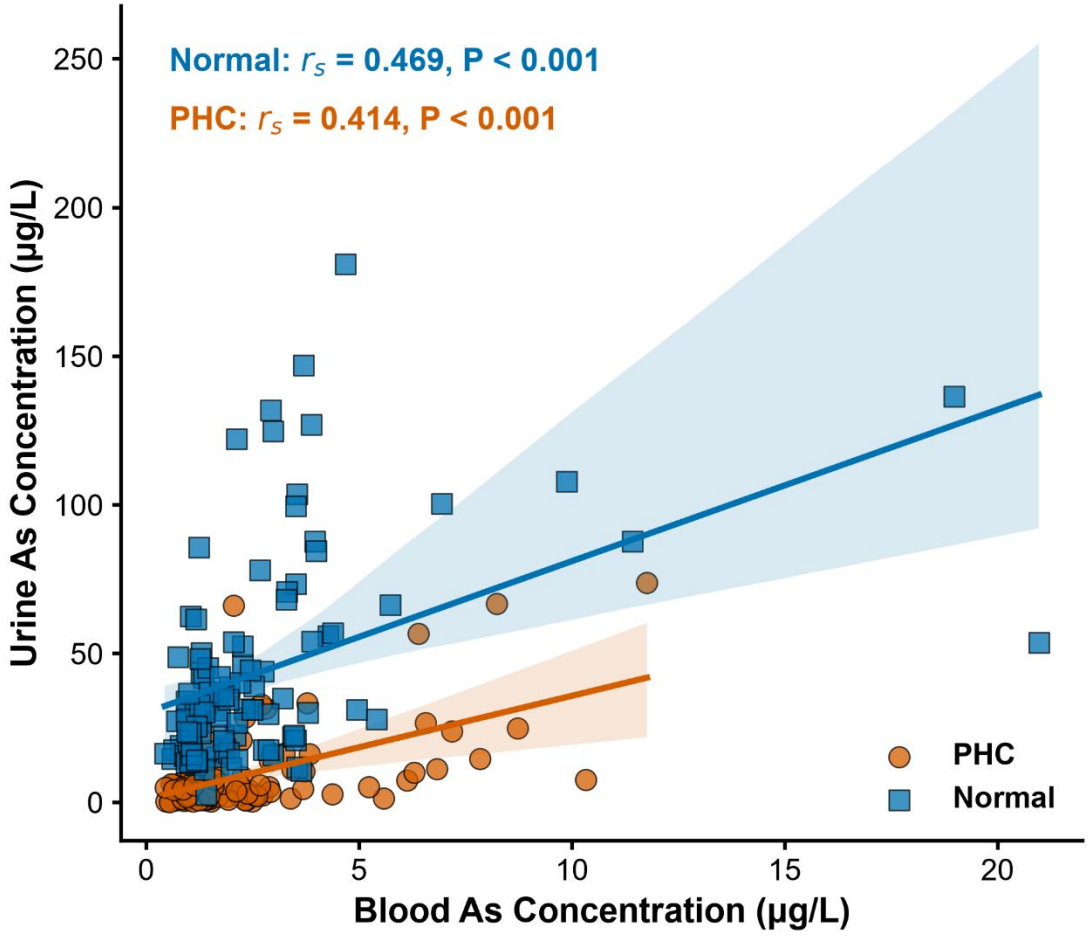


Figure 3. Scatter correlation and linear fitting plot of blood As and urine As in the PHC and normal groups.

NOTE: Scatter plot illustrates the relationship between blood and urine As levels. Blue squares and orange circles represent individual data points for the normal control and PHC groups, respectively. The solid lines indicate the linear regression fit for each cohort, accompanied by shaded regions representing the 95% confidence intervals. Spearman's rank correlation coefficients (rs) and corresponding P-values are displayed at the top left. Both the normal (rs = 0.469, P < 0.001) and PHC (rs= 0.414, P < 0.001) groups exhibited a statistically significant positive

correlation between blood and urinary arsenic levels.

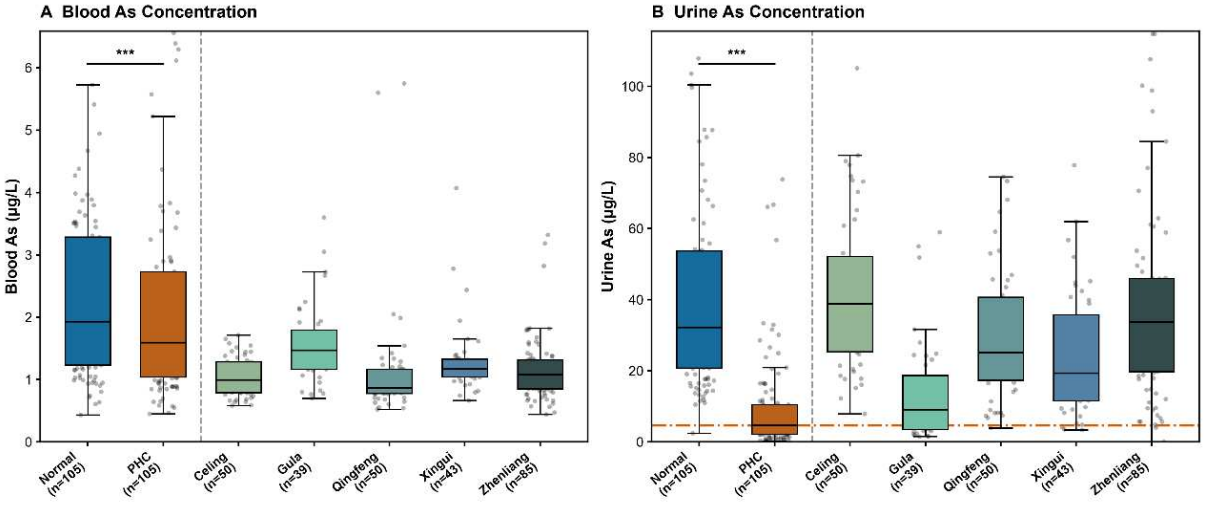


Figure 4. Altered arsenic distribution profiles in PHC patients compared to Normal group and high-background regional populations.

NOTE:(A) Blood arsenic (As) concentrations and (B) urine arsenic (As) concentrations across different groups. The groups include Normal group (Normal), patients with primary hepatocellular carcinoma (PHC), and residents from five reference regions (Celing, Gula, Qingfeng, Xingui, and Zhenliang). The vertical dashed gray line separates the core clinical study groups from the regional environmental reference groups. In Panel B, the horizontal dash-dotted red line indicates the median urine As concentration of the PHC group, highlighting the restricted urinary arsenic excretion in liver cancer patients relative to other populations. Boxplots show the median (thick horizontal line), interquartile range (IQR, box), and whiskers extending to 1.5 × IQR. Individual data points are overlaid as semi-transparent dots to display the sample distribution. Statistical significance between the Normal and PHC groups is denoted by asterisks (** $p < 0.001$). To ensure optimal visualization of the box bodies, extreme upper outliers are not fully displayed on the Y-axis, though they were included in all statistical calculations.

Assessment of PHC interference on bodily fluid biomarkers

As demonstrated by the preceding superimposed analysis of EDI and bodily fluid biomarkers, relying solely on environmental exposure doses is insufficient to accurately characterize the true internal exposure burden of populations. To precisely quantify the perturbation of biomarker concentrations induced by the pathological state of PHC, this section employs Receiver Operating Characteristic (ROC) curves and nested binary logistic regression analyses. Specifically, we evaluated the discriminatory power of blood and urinary As in distinguishing PHC patients from reference populations under two distinct control scenarios: (1) healthy control group vs. PHC case group; and (2) a composite group (Normal + residents from the five high-background villages) vs. PHC case

group. The latter scenario was designed to encapsulate the natural fluctuations of multi-pathway environmental exposures inherent to the karst region.

Prior to adjusting for age and gender, Figure 5 and Table 3 demonstrate that urinary As concentration exhibited highly sensitive discriminatory power for the pathological state of PHC, yielding an impressive Area Under the Curve (AUC) of 0.916 in the first scenario. Even after introducing the confounding noise of natural background exposures in the second scenario, urinary As maintained a robust AUC of 0.866. In stark contrast, because blood As experienced only a marginal decrease in the PHC group, its unadjusted discriminatory performance hovered near random classification, with AUCs of merely 0.555 and 0.638 across the two scenarios, respectively.

Given the objective disparities in age and gender distributions between the Normal group and the PHC group, nested multivariable binary logistic regression models were constructed to eliminate demographic confounding. Base models incorporating only age and gender were initially fitted. Subsequently, blood As and urinary As were individually introduced into the models to quantitatively assess their incremental predictive value.

DeLong's test revealed starkly contrasting characteristics between the two biomarkers. In the first scenario, adding blood As to the already high baseline of the base model yielded no significant incremental benefit. Conversely, the inclusion of urinary As significantly enhanced the model's discriminatory capacity. This trend was further amplified against the complex background of the second scenario, where the integration of urinary As yielded a substantial AUC increment of 0.155 ($P < 0.01$).

Synthesizing the evaluation results from the nested models, it is evident that the distribution of urinary As is profoundly perturbed by the presence of PHC. This severe pathological perturbation effectively masks the environmental signals generated by external multi-pathway exposures, thereby limiting the reliability of urinary As as a standalone biomarker for environmental exposure assessment. In contrast, blood As is only weakly affected by hepatic pathological changes and exhibits no specific diagnostic efficacy for PHC. Consequently, it stably retains and reflects the true characteristics of external environmental exposures. The disparate response characteristics of these bodily fluid biomarkers highlight a critical implication: in regions characterized by the convergence of complex environmental backgrounds and endemic diseases, incorporating blood As monitoring is of paramount practical significance for the accurate assessment of the true human exposure burden.

Table 3. Diagnostic performance of arsenic concentrations in blood and urine for primary hepatocellular carcinoma (PHC).

Group Comparison	*Biomarker*	*AUC*	*95 %CI*	*Best Cut-off*	*Sensitivity*	*Specificity*
Normal vs PHC	Age	0.932	0.90-0.964	53.5	70.9	98.1

	Gender	0.693	0.62-0.77	0.50	76.7	61.9
	As_{urine}	0.916	0.87–0.96	11.55 μg/L	78.1	97.14
	$As_{urine\ (ADj)}$	0.978	0.96–0.99	0.03	100	49.5
	As_{blood}	0.555	0.48–0.63	6.12 μg/L	10.48	95.24
	$As_{blood\ (ADj)}$	0.945	0.92–0.97	0.16	100	77.1
Normal+Regions vs PHC	Age	0.605	0.55-0.66	65.5	63.4	70.9
	Gender	0.702	0.65-0.76	0.5	76.7	63.7
	As_{urine}	0.866	0.82–0.91	12.21 μg/L	80	83.06
	$As_{urine\ (ADj)}$	0.897	0.86–0.93	0.06	96.1	50
	As_{blood}	0.638	0.55–0.68	1.48 μg/L	56.19	70.43
	$As_{blood\ (ADj)}$	0.726	0.67–0.78	0.1	86.4	50

Note: AUC, area under the receiver operating characteristic (ROC) curve. It is vital to state that this analysis quantifies disease-induced biomarker perturbation, not clinical diagnostic potential. The optimal cut-off values were determined by maximizing the Youden's index (Sensitivity + Specificity - 1). Importantly, the unadjusted cut-offs represent actual physiological metrics (years for age, μg/L for arsenic), whereas the adjusted cut-offs (Adj) represent dimensionless predicted probabilities (ranging 0-1) derived from multivariable logistic regression. The "Normal+Regions" group represents a pooled reference population encompassing both Normal group and residents from high-arsenic background regions.

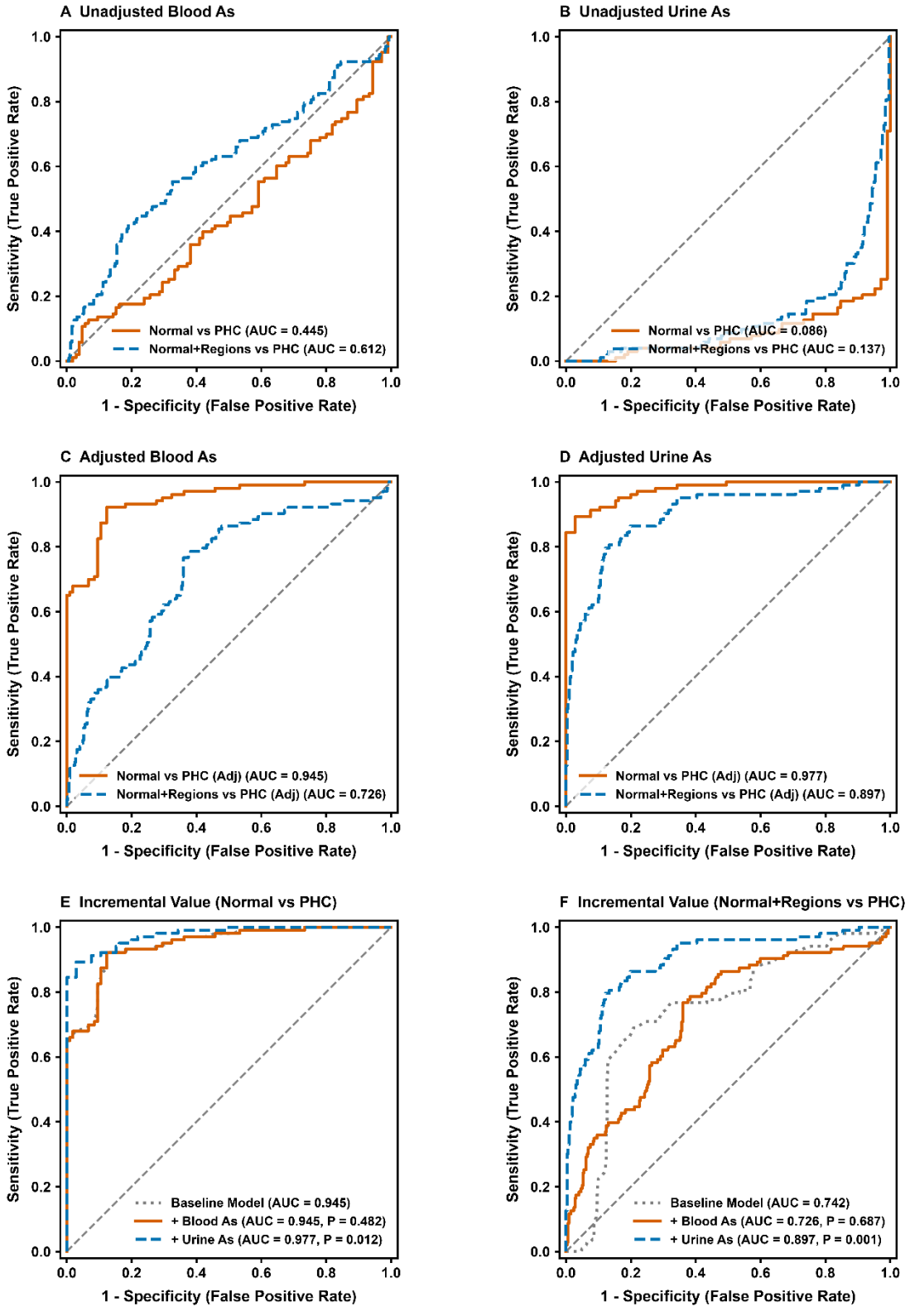


Figure 5. Receiver operating characteristic (ROC) curves evaluating the diagnostic performance of blood and urine arsenic levels for primary hepatocellular carcinoma (PHC).

NOTE: (A) ROC curves for blood arsenic (Blood As) and (B) urine arsenic (Urine As). The solid orange lines indicate the capability to differentiate PHC patients purely from Normal group (Normal vs PHC). The dashed blue

lines represent the performance when discriminating PHC patients from a pooled baseline population, which includes both Normal group and residents from high-arsenic background regions (Normal+Regions vs PHC). The area under the curve (AUC) for each model is annotated in the lower right corner. The diagonal gray dotted line represents the reference line of no discrimination (AUC = 0.50). Notably, Urine As (Panel B) exhibits superior and robust capability to reflect PHC perturbation (AUC > 0.86) irrespective of environmental background exposures, significantly outperforming Blood As (Panel A).

Discussion

Structural characteristics of external environmental exposure in the high-background karst region

Human As exposure in the high-background karst region is characterized by an external exposure structure where rice intake is overwhelmingly dominant, while the contribution from direct soil ingestion is remarkably low. This diet-driven exposure paradigm contrasts sharply with the classic groundwater-driven As exposure models typically observed in South Asia and Latin America [26]. While traditional studies have long regarded As-contaminated groundwater as the primary exposure source, the insidious nature and critical importance of the rice consumption pathway have increasingly been recognized in recent years [27-31]. In the specific context of this karst study area, surface water As concentrations are significantly lower than those in the aforementioned traditional contamination zones, thereby substantially attenuating the water exposure pathway. Conversely, rice serves as the highly frequently consumed staple food for local populations. The superimposition of the high-As soils inherent to the karst region and the exceptional As bioaccumulation capacity of rice culminates in a highly stable dietary As exposure route [30-31]. Previous epidemiological studies conducted in U.S. regions with low drinking-water As have corroborated that populations maintaining high-rice dietary habits exhibit significantly elevated urinary As metabolites of various species [32]. This indirectly substantiates that, in environments where waterborne exposure is restricted, dietary rice acts as the pivotal driver of the internal As burden in human populations.

More crucially, despite the widespread exceedance of soil As standards in the study area, direct exposure via incidental soil ingestion remains exceedingly limited. Instead, As is predominantly mobilized and bioaccumulated through the soil-rice continuum, ultimately utilizing rice as a vehicle to inflict insidious exposure on the human body. These findings profoundly elucidate that food-chain transfer, mediated by agricultural crops, constitutes the fundamental pathway sustaining the high As exposure of populations in this specific environment.

Differential responses of blood and urinary As to multi-pathway exposures in high-background populations

In the complex multi-pathway exposure scenarios of the high-background karst region, despite the total estimated daily intakes (EDIs) being relatively comparable across the villages, the total blood As burden among the populations remained highly stable, whereas total urinary As exhibited pronounced spatial heterogeneity.

This phenomenon is primarily governed by the physicochemical properties of the exposure media coupled with the toxicokinetic characteristics of the human body. Arsenic in water exists predominantly in a free state with high bioavailability; following ingestion, it is metabolized by the liver and rapidly excreted by the kidneys. This rapid excretion mechanism renders total urinary As highly sensitive and indicative of short-term or recent changes in external exposure, though it also makes it highly susceptible to fluctuations [33]. Conversely, the primary exposure source for the population in this region is dietary rice. Constrained by the matrix effect, As within the rice matrix is released slowly in the gastrointestinal tract, leading to a delayed rate of entry into systemic circulation. Under long-term accumulation, this forms a metabolically lagged steady-state pool in the blood, thereby allowing total blood As to maintain exceptionally high stability [34-35].

From the perspective of biomarker application, total urinary As serves as an excellent indicator of external environmental exposure, capable of acutely reflecting local variations in exposure pathways. Meanwhile, total blood As is insulated from short-term environmental fluctuations, representing a long-term baseline of continuous exposure. Therefore, in complex multi-pathway exposure scenarios, relying solely on the highly fluctuant urinary As for exposure assessment may introduce bias due to its inherent spatial heterogeneity. In practical population health biomonitoring, it is recommended to utilize urinary As as a sensitive exposure indicator; however, it is imperative to cross-validate and verify these assessment results using the stable characteristics of total blood As. This dual-biomarker approach is essential to comprehensively and objectively reflect the true internal exposure burden of the population.

Characteristics of bodily fluid As in the primary hepatocellular carcinoma (PHC) population

The present study revealed that the geometric means of blood and urinary As in the PHC group were significantly lower than those in the healthy control group. Notably, urinary As plummeted by approximately 87%, whereas blood As exhibited only a mild decline. This phenomenon of a concurrent decrease in both blood and urinary As among PHC patients is likely co-driven by dual mechanisms: a precipitous reduction in exogenous intake and endogenous metabolic dysfunction.

From the perspective of exogenous input, PHC patients universally experience tumor-associated wasting (cachexia), anorexia (loss of appetite), and strict clinical dietary interventions during hospitalization. Consequently, their As burden derived from daily dietary intake is substantially lower than that of healthy

populations under natural exposure, directly contributing to an overall downward shift in the baseline of total body As.

Regarding endogenous metabolism, correlation analysis demonstrated that blood and urinary As in the PHC group maintained a significant positive correlation ($rs=0.414$, $P<0.001$); however, a mere reduction in intake cannot entirely explain the vast disparity in the magnitude of their declines. Previous studies have established that hepatic parenchymal damage significantly impairs the body's methylation capacity for trivalent arsenic [As(III)], thereby triggering the abnormal retention of trace elements within diseased organs [36-38]. Given that urination is the primary pathway for human As excretion, the drastic reduction in total urinary As strongly suggests that the As excretory channels in PHC patients are severely obstructed, potentially leading to the internal accumulation of unexcreted As within organs.

It is noteworthy that the present study utilized absolute concentrations when evaluating urinary As, without applying urinary creatinine adjustment. The primary rationale is that human creatine synthesis is highly dependent on the liver. When PHC induces severe hepatocellular necrosis, compounded by the muscle wasting (sarcopenia) typically observed in oncology patients, the baseline excretion of urinary creatinine undergoes a pathological decline [39-40]. Therefore, applying creatinine adjustment to urinary As in the liver cancer population could artificially inflate the adjusted urinary As levels.

More crucially, there is a staggering 7-fold disparity in absolute urinary As concentrations between the PHC and healthy groups in this study. Such a massive magnitude of difference far exceeds the natural fluctuation range attributable merely to the urine concentration (hydration) effect [41-43]. Thus, utilizing absolute urinary As concentrations more objectively and authentically substantiates the profound disruption of the As excretory metabolic pathways caused by the pathological state of liver cancer.

Interference of PHC on As biomarkers

In traditional environmental epidemiological assessments, bodily fluid biomarkers are presumed to map external environmental exposure doses in a linear or semi-linear fashion. However, coupled with multi-scenario model deductions, this study profoundly reveals the intense perturbing effect of primary hepatocellular carcinoma (PHC)—a severe underlying disease—on the indicative efficacy of bodily fluid As.

First, the bodily fluid As in PHC patients exhibits a profound interference that overrides environmental exposure signals. Model 1 confirmed that when distinguishing healthy populations from PHC patients, the AUC of urinary As alone reached as high as 0.916. Notably, correlation analysis in the healthy control and high-background natural exposure groups revealed no significant association between demographic factors (age, gender) and total urinary

As. This indicates that under physiological conditions, urinary As is primarily governed by external environmental input. Even when Model 2 introduced the natural fluctuation data of populations with multi-pathway exposures in the high-background region, the AUC of urinary As remained robust at 0.866. In contrast, blood As was minimally perturbed by liver cancer, with its unadjusted AUC hovering around 0.55. These findings suggest that the metabolic derangement triggered by liver cancer is potent enough to override or mask the impacts of external multi-pathway environmental inputs.

Secondly, at the level of the biomarker evaluation framework, liver cancer precipitated a divergence in the indicative efficacy between blood and urinary biomarkers. After stripping away the substantial predictive contributions of demographic characteristics (age and gender), the nested models conversely highlighted the robust stability of blood As. Because blood As exhibited only a mild decrease in the PHC group, its unadjusted AUCs for the disease state hovered near random classification (0.555 and 0.638). Furthermore, it provided almost no incremental predictive value in the nested models, robustly proving that blood As is highly insulated against hepatic pathological remodeling.

Therefore, in exposure regions characterized by a high incidence of major endemic diseases like liver cancer, the application of bodily fluid biomarkers necessitates a combinatorial strategy. The highly fluctuant urinary As can serve as a sensitive indicator to capture abnormal alterations in host metabolism. Simultaneously, the stable total blood As must be utilized to cross-verify the true environmental exposure background. This dual-biomarker approach is crucial to prevent the misjudgment of environmental exposure risks that might arise from pathological or physiological declines in standalone urinary As data.

Study limitations

Despite its findings, this study has several limitations that warrant further investigation. First, as a cross-sectional study, this research can only observe associations among exposures, biomarkers, and disease states; it cannot establish a causal temporal sequence between the progression of liver injury and alterations in As metabolism, which would require a longitudinal cohort design. Second, this study only measured total As levels without conducting As speciation analysis. Consequently, it is unable to distinguish between inorganic As and its methylated metabolites. This precludes the precise quantification of the specific metabolic steps where hepatic methylation is obstructed, and it cannot completely rule out the potential interference of dietary organic As on the total As measurements. Third, to mitigate the influence of high inter-individual variability, the external exposure assessment utilized village-level mean EDIs to characterize the population exposure structure. Therefore, the current study is restricted to group-level trend analyses and cannot derive individual-level dose-response

relationships.

Conclusions

Focusing on the Guangxi karst region, characterized by a high geochemical As background and a co-occurring high incidence of primary hepatocellular carcinoma (PHC), this study demonstrates that dietary rice constitutes the predominant source of As exposure for the local population. Drinking water serves merely as a localized, secondary source, while the direct soil pathway exerts minimal impact. In healthy populations, blood As primarily reflects the long-term baseline of diet-driven continuous As exposure, whereas urinary As exhibits higher sensitivity to waterborne As inputs. Consequently, traditional external exposure assessments based solely on environmental media standards and estimated daily intakes (EDIs) exhibit significant discrepancies from the true internal exposure burden of the population. Crucially, the presence of PHC profoundly suppresses urinary As excretion, whereas blood As remains relatively resilient against malignant hepatic pathological perturbations. Ultimately, this study substantiates that in regions where high environmental As and endemic liver cancer converge, relying exclusively on external exposure metrics or standalone urinary As evaluation paradigms introduces inherent bias. A combinatorial evaluation strategy integrating both blood and urinary As can effectively rectify pathological confounding biases, thereby providing a robust scientific basis for the precise assessment of As exposure in high-risk populations.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The author(s) declare no competing interests.

Ethical Approval and Consent to Participate

This study was conducted in strict accordance with the ethical principles of the Declaration of Helsinki. The human blood data were obtained from the Guangxi Key Research and Development Program project “Multicenter Study on Carcinogenic Effects and Spatial Prediction Model of Heavy Metal Exposure in High-Incidence Areas of Primary Liver Cancer in Guangxi” (Grant No. AB23026041). The study protocol was reviewed and approved by the Medical Ethics Committee of Guangxi Medical University (Approval No.: 2022–0181).

Consent for Publication

Not applicable.

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