Environmental Research 219 (2023) 115030

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Arsenic may be a carcinogenic determinant of a subset of gallbladder cancer: A pilot study

Giuseppe De Palma [a,b,1], Gonzalo Carrasco-Avino [c,d,1], Enrica Gilberti [b], Moris Cadei [e], Tatiana Pedrazzi [a], Ivan M. Gallegos Mendez [c], Alejandra Roman [c], Nicolas Demartines [f], Paolo Boffetta [g,h], Ismail Labgaa [f,*]

[a] Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy
[b] Unit of Occupational Health, Occupational Hygiene, Toxicology and Prevention, ASST Spedali Civili di Brescia, Brescia, Italy
[c] Department of Pathology, Hospital Clinico Universidad de Chile, Santiago, Chile
[d] Department of Pathology, Clinica Las Condes, Santiago, Chile
[e] Pathology Unit, ASST Spedali Civili di Brescia, Brescia, Italy
[f] Department of Visceral Surgery, University Hospital of Lausanne (CHUV), University of Lausanne (UNIL), Switzerland
[g] Stony Brook Cancer Center, Stony Brook University, Stony Brook, NY, USA
[h] Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy

ARTICLE INFO

Keywords:
Exposure
Toxicants
Heavy metals
Carcinogen
Prevention

ABSTRACT

Gallbladder cancer (GBC) is one of the deadliest malignancy and treatment options are deplorably limited. Better strategies of prevention are urgently needed but knowledge on risk factors remains scarce. Recent data suggested that arsenic (As) may be involved in GBC carcinogenesis but the question remains debated. To date, there are no data on As measurement in GBC samples. This pilot study aimed to measure As concentrations in tissue samples from patients with GBC compared to non-cancerous gallbladder (NCGB). Included patients underwent cholecystectomy at Hospital Clinico Universidad de Chile, Santiago in Chile, a country with high As exposure, between 2001 and 2020. Tissue samples were preserved in formalin-fixed, paraffin-embedded blocks. Selected samples were retrieved, processed and submitted to inductively coupled plasma mass spectrometry (ICP-MS) to determine As concentrations. A total of 77 patients were included, including 35 GBC and 42 NCGB. The two groups were comparable, except for age (68 *vs*. 49 years, $p < 0.001$). Measured in 11 GBC and 38 NCGB, total As was detected in 5 GBC (14%) compared to 0 NCGB samples ($p < 0.001$). GBC group also showed higher median values of As compared to NCGB ($p < 0.001$). This pilot study provided a proof-of-concept to measure As concentrations in gallbladder samples and showed higher level of As in GBC samples compared to NCGB, paving the way for future studies aiming to investigate the impact of As on GBC, which may contribute to the prevention of this deadly disease.

1. Introduction

Gallbladder cancer (GBC) is one of the deadliest malignancies, associated with dismal outcomes (i.e. 5-years survival rates ~5%) and limited treatment options (Hundal and Shaffer, 2014; Randi et al., 2006). It is thereby a cancer for which prevention would be of particular value and importance (Jaffee et al., 2017). Unfortunately, its risk factors remain largely unclear, precluding the establishment of targeted and efficient prevention strategies.

Arsenic (As) is a metalloid that can contaminate air, soils, food and drinking water (Bouvard et al., 2009). The geographical distribution of its natural occurrence is heterogeneous, with regions in countries like Chile or Bangladesh showing strikingly high concentrations (Bundschuh et al., 2022). This toxicant has been categorized as an established human carcinogen with acknowledged impact on lung, skin and bladder cancers but no conclusive evidence on digestive malignancies (Bouvard et al., 2009; Humans, 2012). Metabolized by the liver, As-most toxic metabolites are excreted through the bile (Argos et al., 2010; Ponomarenko

* Corresponding author. Department of Visceral Surgery University Hospital of Lausanne (CHUV). Rue du Bugnon 46. CH-1011, Lausanne, Switzerland.
E-mail address: ismail.labgaa@chuv.ch (I. Labgaa).
[1] Co-first authors.

https://doi.org/10.1016/j.envres.2022.115030
Received 4 October 2022; Received in revised form 24 November 2022; Accepted 7 December 2022
Available online 9 December 2022

et al., 2017). A carcinogen effect on liver, bile ducts and gallbladder may hence be postulated (Ganesan et al., 2020; Reyes et al., 2022). Nonetheless, data on its association with GBC are scarce and contradictory (Barahona Ponce et al., 2020; Ganesan et al., 2020; Lee et al., 2020; Madhawi et al., 2018; Tsai et al., 1999). Very few studies measured arsenic in cancer tissue samples, with techniques such as inductively coupled plasma mass spectrometry (ICP-MS). Few studies used fresh biopsies of esogastric cancers (Kohzadi et al., 2017; Nozadi et al., 2021) while only one study utilized FFPE samples for liver cancer (Cano et al., 2021). To date, such data are not available for GBC.

This pilot study aimed to measure and compare As concentrations in tissue samples from patients with GBC *versus* non-cancerous gallbladder (NCGB).

2. Materials and methods

2.1. Study design

This is a retrospective case-control study measuring and comparing As concentration in tissue samples from patients with GBC *versus* NCGB, enrolled at Hospital Clinico Universidad de Chile in Santiago (Chile) between 2001 and 2020. The study was conducted in accordance with the Declaration of Helsinki, and it conformed to good clinical practice guidelines. Research protocol was approved by the Institutional Review Board (#35–2022). The region of recruitment was specifically selected as previous data suggested a correlation between GBC incidence and As concentration in drinking water (Ganesan et al., 2020); Chile is among the countries with the strongest environmental pollution by As. Patients underwent surgery with cholecystectomy for gallbladder cancer (GBC) or non-cancer etiologies (NCGB), such as gallstones and acute cholecystitis. Patients with available tissue samples were included. Final diagnosis was reviewed based on pathological analyzes of the specimen conducted by hepatobiliary pathologists (G.C.A and I.M.G.M.) according to the AJCC guidelines. Variables like age, gender and the presence of gallstones were collected.

2.2. Tissue samples

Tissue samples were stored in formalin-fixed paraffin-embedded (FFPE) blocks at the archives of the Pathology Department of Hospital Clinico Universidad de Chile, at room temperature. Histological slides (H&E) and FFPE blocks were retrieved. Slides were reviewed to confirm the diagnosis and representativeness of each sample. Tissue blocks were cut in order to obtain 150–200 mg of tissue for As levels assessment.

2.3. Quantification of as

First intent was to quantify As species in 28 samples. In every case, analyses were ran blindly as to the case-control status. The analysis was performed with inductively-coupled mass spectrometer (ICP-MS, PerkinElmer, ELAN DRC II) connected to a HPLC (Thermo Separation Products) with Anion Exchange Hamilton PRP-X100 column, 4.1 mm id. x250mm 10 μm, the mobile phase consisting of 2 solutions of ammonium bicarbonate at pH 8.5 and ammonium sulfate 20 mM at pH 7.0. External calibration was performed with a mix of 2 μg/L starting from the salts of the As species: Trivalent oxide arsenic As2O3 (AsIII; PM 197.84); Pentavalent oxide arsenic As2O5 (AsV; PM 229.84); Monomethylarsonic acid (CH2)AsO(OH)2 (PM 184) (MMA); Dimethylarsinic acid C2H7AsO2 (PM 137.99) (DMA); Arsenobetaine (CH3)AsCH2COOH (AsB; PM 258). In this case, deparafinated gallbladder samples were weighed and sonicated in 1 mL of bi-distilled water for 2 h at 60 °C. Accuracy was determined analysings Bovin Liver 1577 b as reference material. The Limit of Detection for all As species was 0.01μg/. The mean imprecision between series, expressed as CV% was: 2.5 for AsIII and AsB and 5.0 for As(V), MMA and DMA.

The remaining 49 samples underwent total As analysis. Gallbladder samples were deparaffinized and dried at 100 °C overnight (ON), then weighed and digested in a solution of 50% Nitric acid and 50% distilled water at a temperature of 70 °C for 1 h. The resulting solution was diluted in double-distilled water. Total As determination was performed by the same ICP-MS instrument with *TotalQuant* determination. Calibration was accomplished with calibration standard 3, multi-element stock (10 g/mL; PerkinElmer). Accuracy was determined by averaging values obtained from analysis of NIST-certified reference material 1643 (trace element in water) and Bovin Liver 1577 b. Accuracy expressed as coefficient of variation (CV%) ranges from 6.5 to 9%. The Limit of Detection is < 0.0005 μg/g. Limit of quantification was calculated from the analysis of 10 blanks based on 3 standard deviations of the background signal.

2.4. Statistical analysis

Categorical variables were provided as frequencies with valid percentages and compared with Fisher's exact test. Continuous data were displayed as median with interquartile range and compared with the Mann-Whitney *U* test. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed with IBM SPSS Statistics, version 27.0.

3. Results

3.1. Cohort

The cohort included 35 GBC and 42 NCGB patients from Chile, for whom tissue samples were retrieved and analyzed by ICP-MS. Table 1 summarizes the main characteristics of the cohort. While the two groups were comparable for gender and gallstones prevalence, GBC patients were older (68 *vs.* 49 years, $p < 0.001$). GBC group had a majority of women (74.3%), with a median age of 68 years (58–72) and a high prevalence of gallstones (86.2%).

3.2. Measurement and comparison of as concentrations in tissue samples of GBC versus NCGB

As species –AsIII, AsV, MMA, DMA and AsB- were measured in 24 and 4 GBC and NCGB patients, respectively. These species were not separately detected, in any patient. The remaining samples were utilized to measure total As in 11 GBC and 38 NCGB patients. Total As level was detected above the limit of the quantification method in 5 GBC patients but not in any NCGB ones (14.3 *vs.* 0%, $p < 0.001$). Comparison of the median values also confirmed a significant difference between the groups, with higher concentrations of As in GBC tissues ($p < 0.001$) (Fig. 1).

3.3. Characterization of cases with detectable levels of as

The 5 GBC cases with detectable levels of As were all females with mean age of 59 years old (45–72). Three of them were born in the Metropolitan (central zone), one in the Valparaiso (central zone) and one in the Maule (south-central zone) regions of Chile.

Histologically, we reported 4 adenocarcinomas: one well, two moderately-differentiated, 1 NOS (not otherwise specified) and 1 adenosquamous carcinoma. Three of them had gallstones.

To date, two of them are still alive while the 3 other patients died

Table 1
Cohort.

	GBC (n = 35)	NCGB (n = 42)	p-value
Gender (female)	26 (74.3)	32 (76.2)	1.000
Age (years)	67.5 (57.8–72)	49 (40–64)	<0.001
Gallstones	25 (86.2)	40 (95.2)	0.218

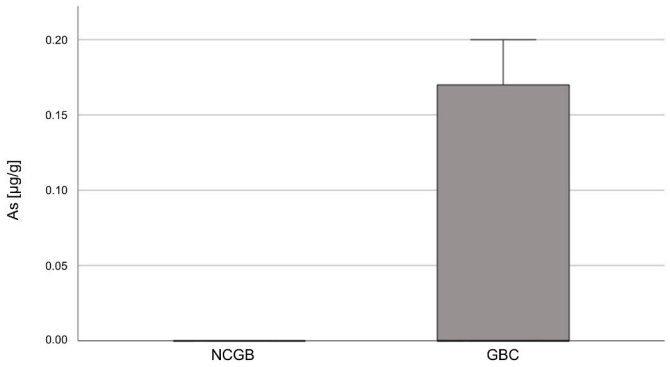

Fig. 1. As levels in GBC and NCGB, Boxplots showing the concentrations of arsenic (As [µg/g]) in tissue samples from gallbladder cancer (GBC) and non-cancerous gallbladder (NCGB).

from GBC.

4. Discussion

This study provided a proof-of-principle to measure As levels in gallbladder specimen and detected higher levels of As in GBC samples compared to controls, in a cohort of patients from Chile.

GBC is characterized by an extremely aggressive biology. Dramatically, GBC patients are often diagnosed at advanced stage where curative treatments are no longer possible. For the small subset of patients who may receive curative therapies (i.e. only around 10%), complex and multi-disciplinary management are required and surgery remains the cornerstone of treatment, associated with a substantial risk of morbidity and of recurrence (Cotter et al., 2022; Hundal and Shaffer, 2014). Important efforts have been pursued to improve outcomes of these patients, but with mitigated success (Cotter et al., 2022; Kanthan et al., 2015). These elements illustrate to which extent efficient prevention measures would be paramount for this deadly disease. However, such measures require the robust identification of risk factors, which remains a critical unmet need. The geographical and socio-economic distribution of GBC is uniquely heterogeneous and therewith intriguing. Outliers countries/regions ranking on the top of the list for GBC incidence are depicted on each continent: Chile, Bangladesh, Poland, Alaska or Ghana (Gamboa and Maithel, 2020). Although GBC likely results from multi-factorial effects, one common factor of these countries/regions is their exposure to As (Bundschuh et al., 2022; Gamboa and Maithel, 2020; Ganesan et al., 2020). In fact, the correlation between GBC incidence and As level in drinking water was already shown worldwide, as well as in India and Taiwan (Ganesan et al., 2020). Similar data also suggested a carcinogenic impact of As on bile duct, leading to an increased risk to develop cholangiocarcinoma (Reyes et al., 2022). Recent findings exemplified the complexity of this question. A recent study conducted in China analyzed a large cohort of patients including 259 GBC and 701 gallstone cases and 851 controls in whom a panel of 18 metals were measured in serum (Lee et al., 2020). As was identified as a protective factor against GBC. In response to these surprising results, a group of European researchers conducted Mendelian randomization analysis, integrating genomic factors such as variants of *AS3MT*, a gene involved in As metabolism (Barahona Ponce et al., 2020). Interestingly, this approach allowed dissecting the effect of each As species (i.e. inorganic As (iAs) monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA)), and confirmed a protective role of iAs and MMA but highlighted a deleterious effect of DMA. Of note, DMA is excreted through the bile while MMA is preferentially excreted in urines. Studies investigating the potential link between exposure to As and GBC remain rare and showed contradictory results, feeding the debate. In addition, available reports were mostly ecological studies with rare data providing As measurement on an individual basis. Therefore, measuring As in tissue samples appears as a pertinent strategy but it requires demanding techniques, particularly for specimen stored in FFPE. Recently, a successful attempt showed the feasibility to measure heavy metals like As in tissue samples of cancer specimen stored in FFPE -utilizing ICP-MS (Cano et al., 2021)- but no data are available for GBC, so far.

The present report was designed as a pilot study aiming to provide initial evidence and to continue exploring this promising breach of data suggesting an impact of As in GBC. Consequently, it inevitably displays limitations that must be addressed. Beside the modest sample size that precluded from conducting more sophisticated analysis, ICP-MS technique could not detect the different As species. This is a point deserving to be specifically tackled by future studies, considering the results generated by Barahona Ponce et al. (Barahona Ponce et al., 2020). Nonetheless, total As was detected in a significant subset of analyzed GBC samples (14%), but not in any controls. Several hypotheses could be formulated. In particular, As may be a determinant of GBC in a subgroup of GBC patients exposed to higher level of As, or genetically more susceptible to its carcinogenic effects. Synergic effect may be another hypothesis; in other words, the effect of As in biliary cancers may be potentiated by other risk factors like gender or gallstones. The limited sample size also precludes form any definitive conclusion for a clear epidemiological association. Also, confounding by age remains a potential source of bias in our analysis. However, the small number of subjects with total As level above 0 precluded a formal assessment of it.

This pilot study detected higher levels of As in GBC samples compared to controls, providing novel data supporting the carcinogenic effect of As in a subset of GBC patients. It paves the way and also stresses the need for future studies aiming to further unveil this question, integrating the analysis of the various As species as well as genomic factors.

Funding

None.

Availability of data and materials

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors contributions

Study concept and design: GDP, GCA, PB, IL: Acquisition of data: GDP, GCA, IMGM, AR, EG, MC, TP: Analysis and interpretation of data: GDP, GCA, IL: Drafting of the manuscript: GDP, GCA; IL: Critical revision of the manuscript for important intellectual content: GDP, GCA, EG, MC, TP, IMGM, AR, ND, PB, IL.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Not applicable.

References

Argos, M., et al., 2010. Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. Lancet 376, 252–258.

Barahona Ponce, C., et al., 2020. Arsenic and gallbladder cancer risk: Mendelian randomization analysis of European prospective data. Int. J. Cancer 146, 2648–2650.

Bouvard, V., et al., 2009. A review of human carcinogens–Part B: biological agents. Lancet Oncol. 10, 321–322.

Bundschuh, J., et al., 2022. Global arsenic dilemma and sustainability. J. Hazard Mater. 436, 129197.

Cano, L., et al., 2021. Metallomic profile in non-cirrhotic hepatocellular carcinoma supports a phenomenon of metal metabolism adaptation in tumor cells. Sci. Rep. 11, 14195.

Cotter, G., et al., 2022. Using Machine Learning to Preoperatively Stratify Prognosis Among Patients with Gallbladder Cancer: a Multi-Institutional Analysis. HPB, Oxford).

Gamboa, A.C., Maithel, S.K., 2020. The landmark series: gallbladder cancer. Ann. Surg Oncol. 27, 2846–2858.

Ganesan, N., et al., 2020. Exploring the potential carcinogenic role of arsenic in gallbladder cancer. Eur. J. Cancer Prev. 29, 100–109.

Humans, I. W. G. o. t. E. o. C. R. t., 2012. Arsenic, metals, fibres, and dusts. IARC Monogr. Eval. Carcinog. Risks Hum. 100, 11–465.

Hundal, R., Shaffer, E.A., 2014. Gallbladder cancer: epidemiology and outcome. Clin. Epidemiol. 6, 99–109.

Jaffee, E.M., et al., 2017. Future cancer research priorities in the USA: a lancet oncology commission. Lancet Oncol. 18, e653–e706.

Kanthan, R., et al., 2015. Gallbladder cancer in the 21st century. JAMA Oncol. 2015, 967472.

Kohzadi, S., et al., 2017. Evaluation of trace element concentration in cancerous and non-cancerous tissues of human stomach. Chemosphere 184, 747–752.

Lee, M.H., et al., 2020. A metallomic approach to assess associations of serum metal levels with gallstones and gallbladder cancer. Hepatology 71, 917–928.

Madhawi, R., et al., 2018. Geographical pattern of carcinoma gallbladder in Bihar and its association with river Ganges and arsenic levels: retrospective individual consecutive patient data from Regional Cancer Centre. South Asian J Cancer 7, 167–170.

Nozadi, F., et al., 2021. Association between trace element concentrations in cancerous and non-cancerous tissues with the risk of gastrointestinal cancers in Eastern Iran. Environ. Sci. Pollut. Res. Int. 28, 62530–62540.

Ponomarenko, O., et al., 2017. Selenium-mediated arsenic excretion in mammals: a synchrotron-based study of whole-body distribution and tissue-specific chemistry. Metallomics 9, 1585–1595.

Randi, G., et al., 2006. Gallbladder cancer worldwide: geographical distribution and risk factors. Int. J. Cancer 118, 1591–1602.

Reyes, Darrian; Ganesan, Nivetha; Boffetta, Paolo; Labgaa, Ismail. Arsenic-contaminated drinking water and cholangiocarcinoma. European Journal of Cancer Prevention: January 17, 2022, doi: 10.1097/CEJ.0000000000000740.

Tsai, S.M., et al., 1999. Mortality for certain diseases in areas with high levels of arsenic in drinking water. Arch. Environ. Health 54, 186–193.