RESEARCH COMMUNICATION

Chronic Heavy Metal Exposure and Gallbladder Cancer Risk in India, a Comparative Study with Japan

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Abstract

Background: High incidence of gallbladder cancer (GBC) is reported from North India, with elevated concentrations of heavy metals in water and soil. This Indo-Japan collaborative study compared presence of heavy metals in gallbladder tissues. Methods: Heavy metal concentrations were estimated in Indian GBC and cholecystitis tissues and compared with Japanese GBC and cholecystitis tissues. Spectrophotometry was done for 13 Indian gallbladder tissues (8 GBC, 5 cholecystitis) and 9 Japanese (5 GBC, 4 cholecystitis). Transmission electron microscopy (TEM) thin foil element analysis was done in 10 Indian samples (6 GBC, 4 cholecystitis). Results: Chromium, lead, arsenic and zinc were significantly high in Indian GBC compared with Japanese GBC. Chromium, lead and arsenic were significantly high in the Indian cholecystitis tissues compared to the Japanese. TEM of Indian tissues demonstrated electron dense deposits in GBC. Conclusion: Heavy metals- chromium, lead, arsenic and zinc are potential carcinogens in Indian GBC from endemic areas. This preliminary study links presence of heavy metals in gallbladder cancer tissues in endemic areas.

Keywords: Gallbladder cancer - heavy metals - chemical carcinogenesis - India - Japan

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Introduction

Gallbladder cancer (GBC) has high incidence in certain world populations (Randi et al., 2006). GBC is 8 times more in north India especially in females than in south India (Dhir & Mohandas, 1999). After geographic mapping of 773 GBC at Tata Memorial Hospital, Mumbai (1990-95), Jagannath and colleagues found maximum incidence in Uttar Pradesh, Bihar, West Bengal and Assam (Jagannath et al., 2000). High content of heavy metals in water and sediments in river Ganges downstream was reported (Singh et al., 2005). Pandey (2006) reviewed publications on the role of environmental pollutants on gallbladder carcinogenesis and observed that though a number of heavy metals like nickel, cadmium, etc. have been implicated, the evidence is not robust enough to conclude their association. A recent survey from rural Gangetic basin, North India, cluster analysis revealed a positive correlation of nickel, cadmium and chromium in water with high prevalence of gallbladder diseases in adjacent villages in Vaishali district, Bihar (Unisa et al., 2011).

This is the first study to compare heavy metal levels in the gallbladder tissues in GBC patients from the Gangetic basin in India with Japanese GBC.

Materials and Methods

Gallbladder tissue analysis

Metal contents in 13 fresh tissue samples from Indian patients (8 GBC and 5 cholecystitis) and 9 tissue samples from Japanese patients (5 GBC and 4 cholecystitis) were initially evaluated in the spectrophotometric assessment. Of these 10 Indian samples (6 GBC; 4 cholecystitis) found suitable for study, a transmission electron microscopic (TEM) study including thin foil element analysis for 3 cases was carried out to detect heavy metals in the GBC or in the cholecystitis.

Spectrophotometric Assessment

The fresh tissue samples from GBC and cholecystitis after histological confirmation by frozen section were stored at minus 80°C and subsequently subjected to freeze drying. Freeze dried specimens were washed with acetone and then with 0.01% Triton solution. The washed specimen was mixed in 10 mL of 6.25% tetra methyl ammonium hydroxide (TMAH, Tama Chemical, Japan) with 50 μL of 0.1% gold solution (SPEX Certi Prep.), and then dissolved at 75 degrees centigrade with shaking for 2 hours. After cooling the solution to room temperature and adjusting its volume gravimetric, the obtained solution

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was used for mineral analysis. The mineral concentration was measured with inductive coupled plasma mass spectrometry (ICP-MS; Agilent-7500).

Electron Microscopy

For TEM the fresh tumor tissues of GBC resected from patients were fixed with 2.5% glutaraldehyde. The fixed tissues were dehydrated in serial concentrations of ethanol and subsequently pure ethanol. The tissues were then treated with propylene oxide and Epon mixture (soaked overnight) and embedded in capsules of EM with Epon mixture at 60°C for 24 hours. The sections were examined with a JEM-2000EX electron microscopy. The gold grid was used in the TEM study to avoid false positive results in thin foil element analysis, as gold was the least expected metal to be detected as deposit in any study.

For heavy metal analysis gallbladder tissues were taken from endemic area in India – GBC 8 (2Males, 6Females) and cholecystitis 5 (2M, 3F); and from Japan GBC 5 (2M, 3F) and cholecystitis 4 (2M, 2F)

Statistical analysis

Non-parametric test (Mann-Whitney) was used for comparison between India and Japan for GBC and cholecystitis cases and also between GBC and cholecystitis groups separately for each country. The p value < 0.025 was considered significant after correcting for multiple comparison.

Results

Gallbladder tissue analysis

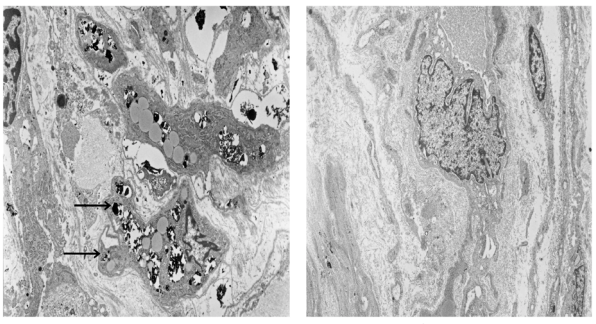
A spectrophotometric assessment for heavy metals of gallbladder tissue samples using freeze dried fresh tissues detected cadmium, chromium, lead, arsenic, mercury and zinc as heavy metals (Table 1). Indian GBC samples (8) from endemic area showed significantly more chromium, lead, arsenic and zinc compared with 5 Japanese GBC samples ($p < 0.025$). Also 5 Indian endemic cholecystitis samples showed significantly more chromium, lead and arsenic compared to 4 Japanese cholecystitis samples.

TEM of 10 tissue samples from India (6 cases of GBC and 4 cholecystitis specimens) was done. Electron dense deposits were present in the stroma in all 6 carcinoma tissue samples (Figure 1a). These deposits were not seen in the stroma of cholecystitis tissue samples (Figure 1b). However, thin foil TEM element analysis of these deposits in 3 cases did not reveal chromium, lead and arsenic but only zinc. The deposits showed mostly metal complexes of iron, zinc and sulfur; and metal compounds Al, Si, S, Ca, F, K, and Zn.

Table 1. Quantitative Analysis of Heavy Metal Content (µg/g) in Indian and Japanese Gallbladder Cancer and Cholecystitis Tissue Samples.

	Gallbladder Cancer				Cholecystitis				P value
	N	Min	Max	Med	N	Min	Max	Med	
Chromium (Cr)									
India	8	0.8	8.2	1.5	5	1.3	22	6.1	0.13[c]
Japan	5	0.1	0.3	0.2	4	0.6	1.1	0.7	*0.02[d]
P value				*0.002[a]				*0.016[b]	
Cadmium (Cd)									
India	8	0	7	0.2	5	0.1	0.3	0.2	0.9[c]
Japan	5	0.0	1.5	0.1	4	0.2	1.3	0.4	0.2[d]
P value				0.8[a]				0.19[b]	
Lead (Pb)									
India	8	0.1	4.8	0.6	5	0.3	2.1	0.8	0.7[c]
Japan	5	0.0	0.1	0.1	4	0.1	0.2	0.1	0.03[d]
P value				*0.002[a]				*0.016[b]	
Arsenic (As)									
India	8	0.5	4300	4.8	5	0.5	2700	1.2	0.2[c]
Japan	5	0.0	0.03	0.0	4	0.03	0.1	0.1	0.3[d]
P value				*0.002[a]				*0.016[b]	
Zinc (Zn)									
India	8	32	250	110	5	30	170	100	0.8[c]
Japan	5	11	21	15	4	25	49	40	0.02[d]
P value				*0.002[a]				0.11[b]	
Mercury (Hg)									
India	8	0	0.1	0.02	5	0.01	0.1	0.03	0.7[c]
Japan	5	0.005	0.02	0.01	4	0.005	0.3	0.02	0.3[d]
P value				0.28[a]				0.29[b]	

* Significant at p<0.025, [a]Comparison between India & Japan for GBC cases, [b]Comparison between India & Japan for Cholecystitis cases, [c]Comparison between GBC and Cholecystitis for India , [d]Comparison between GBC and Cholecystitis for Japan



a b

Figure 1. a Transmission Electron Microscopy of Tissue Samples Showed Electron Dense Deposits (arrow) in The Stroma in all 6 Indian GBC Tissue Samples. b These Deposits Were Not Seen in Indian Cholecystitis Samples.

Discussion

Biliary concentrations of heavy metals cadmium, chromium, and lead were found significantly higher in patients with GBC than in those with gall stones (Shukla et al., 1998). Various risk factors like cholelithiasis, obesity, reproductive factors, and chronic infections of the gallbladder were proposed in the pathogenesis of GBC (Lazcano-Ponce et al., 2001). Heavy metals chromium, cadmium and lead have been implicated in human carcinogenesis (Leonard and Lauwerys, 1980; Silbergeld et al., 2000; Waisberg et al., 2003; Marshall et al., 2007). A recent study in the rural Gangetic basin of North India, cluster analysis identified a positive correlation of nickel, cadmium and chromium in water with high prevalence of gallbladder diseases (Unisa et al., 2011).

It was reported that compared to cholecystitis patients, GBC patients showed high copper, nickel and low zinc and selenium in serum; high cadmium, nickel and low zinc in bile; high chromium in tissue (Singh et al., 2007).

Studies on bile, gallstones suggest the role of heavy metals to GBC but are only indirect evidence. Demonstration of heavy metals in cancer tissue is a more specific proof. Present study using spectrophotometry of diseased gallbladder tissues revealed that the Indian GBC samples showed high levels of chromium, lead, arsenic and zinc ($p < 0.025$), compared to Japanese GBC samples. Also, the Indian cholecystitis samples showed high levels of chromium, lead and arsenic ($p < 0.025$) compared to Japanese cholecystitis samples. High concentrations of heavy metals both in the Indian endemic GBC tissue as well as endemic nonmalignant cholecystitis, in our study suggest a role of heavy metals in carcinogenesis of GBC.

Our initial results were presented at the 8th World Congress 2008 of IHPBA (Chhabra et al., 2008). Electron dense deposits were found in stroma of Indian GBC tissues by TEM. Their absence in Japanese gallbladder cancer tissues and in Indian cholecystitis tissues suggests a role of chromium, lead, arsenic and zinc in carcinogenesis of Indian gallbladder cancers. But thin foil TEM element analysis performed in 3 GBC cases could not localize any of the above metals in the deposits except zinc. It is possible that heavy metals are not part of the deposits, but distributed diffusely in the GBC tissue. Further study is necessary to determine their exact location in the cancer tissue. Interactions with proteins (e.g., with zinc finger structures) are invoked for metal carcinogenicity than binding to DNA (Beyersmann & Hartwig, 2008).

In our study chromium, lead, arsenic and zinc were found in higher concentrations in gallbladder cancer tissues from India compared to Japan. Biliary concentrations of heavy metals cadmium, chromium, and lead were found significantly higher in patients with GBC than in those with gall stones (Shukla et al., 1998). Cluster analysis in a recent study in the rural Gangetic basin of North India, identified a positive correlation of nickel, cadmium and chromium in water with high prevalence of gallbladder diseases (Unisa et al., 2011). The specific importance of carcinogenic mechanisms of chromium, lead, arsenic and zinc in gallbladder cancer needs further study.

Heavy metals could be causal factors or cofactors for carcinogenesis in an inflamed gallbladder. There was no significant difference in heavy metal concentrations between cancer tissues and cholecystitis tissue in Indian samples. The small sample size could account for it. Further, transformation to malignancy depends on duration and degree of exposure to metals. Hence role of chromium, arsenic, zinc and lead in the GBC tissues is to be studied.

Water-soluble chromates are more potent carcinogens - zinc chromate, calcium chromate and chromium trioxide (Langard, 1990). A high tissue concentration of chromium and low manganese was reported in GBC compared to cholelithiasis (Singh et al., 2007).

Our observations revealed significantly high levels of chromium in the Indian GBC and Indian cholecystitis tissue. Leonard & Lauwerys (1980) reported on carcinogenicity and mutagenicity of chromium. Lead content was found significantly high in the Indian GBC compared to Japanese GBC. This supports an early report on lead as carcinogen (Silbergeld et al, 2000).

Cadmium is implicated in carcinogenesis (Waisberg et al,2003), but we did not find significantly high levels of cadmium in GBC.

Arsenic was linked to incidence of cancer (Marshall et al., 2007). In our study arsenic levels were significantly higher in Indian GBC than in Japanese GBC; and in Indian cholecystitis than in Japanese cholecystitis. But Indian GBC compared to Indian cholecystitis showed only slightly higher arsenic values but not significant. Lower zinc levels were reported in patients with GBC (Singh et al., 2007). But our study showed significantly elevated levels of zinc in Indian GBC compared to Japanese GBC.

In conclusion, this study comparing cancer tissue samples of Indian GBC with Japanese GBC showed significantly higher levels of heavy metals Cr, Pb, As and Zn in Indian GBC. These heavy metals may be possible carcinogens in Indian GBC from endemic areas. The specific cause and relationship of these potential carcinogens needs further evaluation.

References

Beyersmann D, Hartwig A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. *Arch Toxicol*, **82**, 493-512.

Chhabra D, Oda K, Utsunomiya H, et al (2008). Heavy metals in gallbladder cancer tissues: a preliminary study. *HPB*, **10 Suppl**, 14.

Dhir V, Mohandas KM. (1999). Epidemiology of digestive tract cancers in India IV. Gallbladder and pancreas. *Indian J Gastroenterol*, **18**, 24-8

Jagannath P, Dhir V, Mohandas KM. (2000). Geographic patterns in incidence of gallbladder cancer in India and the possible etiopathological factors. *HPB*, **2**, 168-9

Langard S. (1990). One hundred years of chromium and cancer: a review of epidemiological evidence and selected case reports. *Am J Ind Med*, **17**, 189-215.

Lazcano-Ponce EC, Miquel JF, Munoz N, et al (2001). Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer J Clin*, **51**, 349-64.

Leonard A, Lauwerys RR. (1980) Carcinogenicity and mutagenicity of chromium. *Mutat Res*, **76**, 227-39

Marshall G, Ferreccio C, Yuan Y, et al. (2007). Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. *J Natl Cancer Inst*, **99**, 920-8.

Pandey M. (2006) Environmental pollutants in gallbladder carcinogenesis. *J Surg Oncol*. **93**, 640-3.

Randi G, Franceschi S, La Vecchia, C. (2006). Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer*, **118**, 1591-602.

Shukla VK, Prakash A, Tripathi BD et al. (1998). Biliary heavy metal concentrations in carcinoma of the gallbladder: case ¬control study. *BMJ*, **317**, 1288-9

Silbergeld EK, Waalkes M, Rice JM (2000). Lead as a carcinogen: experimental evidence and mechanisms of action. *Am J Ind Med*, **38**, 316-23

Singh MP, Singh SP, Shukla VK (2007) Metals in gallbladder carcinogenesis. *ANZ J Surg*, **77 Suppl**, 40.

Singh VK, Singh KP, Mohan D. (2005). Status of heavy metals in water and bed sediments of river Gomti -a tributary of the Ganga River, India. *Environ Monit Assess*, **105**, 43-67.

Unisa S, Jagannath P, Dhir V, et al (2011). Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North

India. *HPB (Oxford)*, **13**, 117-25.
Waisberg M, Joseph P, Hale B, et al. (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, **192**, 95-117.