Serum Levels of Heavy Metals in Cholangiocarcinoma Patients from the Nile Delta Region of Egypt: A Single Centre Study

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ABSTRACT

Background: In Egypt, newly diagnosed cases of cholangiocarcinoma are increasing annually. Several reports from Nile Delta show high levels of heavy metals and organochlorine pesticides in the soil and water. We assess serum levels of Heavy metals [Zinc (Zn), lead (Pb), Cobalt (Co), Cadmium (Cd), Chromium (Cr) and Iron (Fe)] as markers of exposure in cholangiocarcinoma patients and healthy controls from the Nile Delta in Egypt and its correlation with tumor differentiation and tumor marker CA 19-9.

Methods: We included 45 cholangiocarcinoma patients and 20 healthy controls. All patients and controls were permanent residents of North Delta region and were recruited before receiving chemotherapy or radiotherapy, with no restrictions based on age, sex, or tumor stage.

Results: The serum levels of Zn, Pb, Co, Cd and Fe were significantly higher in cholangiocarcinoma patients than controls ($P = 0.001$). A significant higher serum level of Pb was found in patients with well differentiated to moderately differentiated to undifferentiated tumors ($P < 0.05$). Cd has a positive correlation with CA19-9 and negative correlation with patients' survival ($P < 0.5$, $P < 0.01$ respectively).

Conclusions: Cholangiocarcinoma in the Nile Delta region is significantly associated with high serum levels of heavy metals especially Cadmium and lead.

Key words: cholangiocarcinoma, Nile Delta region, heavy metals, lead, Cadmium

INTRODUCTION

Cholangiocarcinoma is a neoplasm arising from the intra- or extra-hepatic bile duct lining epithelium (1). Cholangiocarcinoma represents less than 2% of human malignancies (2). However, it is the second most common primary hepatic malignancy. Many carcinogens have already been identified and the relevant information with regard to these agents is available. One example is the potentially harmful presence of heavy metals that can cause serious health

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problems. The heavy metals in drinking-water pose the greatest threat to public health in this regard (3).

In Egypt, Nile River water is seriously contaminated with heavy metals, pesticides and hydrocarbons as a result of increasing discharge of untreated industrial wastes and agricultural irrigation wastewater (4). Several reports on Dakahlia Province show high levels of heavy metal and organochlorine pesticides in the soil and water in this region (5-7). High concentrations of heavy metals, including cadmium, are among the pollutants in the water. Plants and fish grown in this water are also contaminated with heavy metals (8,9), which can in turn accumulate in humans and animals that feed on these contaminated foods (10). The serum cadmium levels of residents of Dakahlia Province are almost 10 folds higher than those of residents from cadmium-polluted areas in Cairo and 32 times higher than reference levels for healthy populations in the United States (10-12).

The aim of this study was to assess serum heavy metal levels as markers of exposure in cholangiocarcinoma patients and non-cancer comparison subjects from the same region in Egypt and their correlation with the differentiation of cholangiocarcinoma and tumor marker CA 19-9.

MATERIALS AND METHODS

The study included 45 the patients who were newly diagnosed with cholangiocarcinoma from the Gastrointestinal Surgery Center, Mansoura University. All patients were permanent residents of the Nile Delta namely Dakahlya, El Gharbia and Kafr El-Sheikh Governorates. Cholangiocarcinoma was confirmed by reviewing the histopathologic slides of all patients and recruited before receiving chemotherapy or radiotherapy. There were no restrictions based on age, sex, or tumor stage. In addition, 20 control patients were recruited. The control group was chosen by systematic random sampling from patients admitted for surgeries related to non-chronic illness. After the nature of the study had been fully explained, informed written consent was obtained from each patient in the study.

Sample collection

For each patient, 10 mL of blood was collected into a sterile non-heparinized Vacutainer tube. The sample was allowed to clot for 5-10 min and then centrifuged for 10 min at 13,000 rpm. At least 2 mL of clear serum from each sample was transferred to glass tubes, which was labeled with patient name and identification number, clinic name, and collection time and date.

Samples were kept in polyethylene containers that were cleaned using multistep acid-leaching and frozen immediately at -20°C until analysis. Digestion of samples was done using advanced microwave digestion system "Milestone ETHOS-1 lab station with easy-WAVE/easy-CONTROL HPR1000/105 high pressure segmented rotor". The concentrations of cadmium (Cd), iron (Fe), copper (Co), lead (Pb) and zinc (Zn) in both urine and tissue samples were quantified using ICP-spectrometer (iCAP 6000 Series; Thermo Scientific) according to manufacturer instructions. The standards for calibration of the following heavy metals (Cd, Fe, Co, Pb and Zn) were purchased from Sichuan Xinju Mineral Resource Development Stock Co., Ltd. The results were reported in mg/L.

Statistical Analysis

Data was analyzed using SPSS version 16. Qualitative variables were presented as number and per cent. Chi-square test was used for comparison between the two groups. Serum levels of heavy metals were tested for normality distribution using Shapiro test and found to be non-parametric in distribution. They were presented as median and range. Mann-Whitney U test was used for two groups comparison and Kruskal-Wallis test was used for three groups comparison. Spearman (rank) correlation coefficient was used to calculated correlation between variables in the cholangiocarcinoma patients. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Basic Characteristics

Table 1 shows the basic characteristics of the study patients. Cholangiocarcinoma patients were inhabitants of Delta region [24 patients (53.3%) from Dakahlia – 10 patients (22.2%) from KafrElsheikh – 11 patients (24.4%) from Elgharbia].

There were statistically significant differences in the serum levels of Zn, Pb, Co, Cd and Fe between cholangiocarcinoma group and non-cholangiocarcinoma group. On the other hand, there was no statistically significant difference in the serum level of Cr between both groups.

Relationship to Lymph Nodes Involvement

Table 2 shows that the median value of serum levels of Zn, Pb, Cd Fe and Cr which were higher in patients with cholangiocarcinoma having negative LN involvement than in cholangiocarcinoma patients having positive LN involvement but the differences in the serum levels between both groups were statistically significant in levels of Pb, Cd, and Cr only.

Table 1 - Characteristics of the study population [n (%)]

	Cholangiocarcinoma	Controls	Significance test
Age (years)	54.2±7.9	52.5±8.99	t=0.8 P=0.4
Sex			
Male	22 (48.9 %)	8 (40.0 %)	$\chi^2=0.44$
Female	23 (51.1%)	12 (60.0%)	P=0.5
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Positive	21 (46.77%)	0 (0%)	
Negative	24 (53.3%)	20 (100%)	
Zn	0.341(0.02-0.92)	0.106(0.002-0.55)	Z=3.05, P=0.002
Pb	1.16(0.067-2.98)	0.353(0.019-0.932)	Z=4.79, P≤0.001
Co	4.304(0.224-14.1)	0.3255(0.03-2.834)	Z=5.88, P≤0.001
Cd	0.211(0.0-0.951)	0.0091(0.002-0.11)	Z=5.53, P≤0.001
Fe	1.991(0.662-5.774)	0.9565(0.0231-2.652)	Z=3.89, P≤0.001
Cr	0.7063(0.0-2.03)	0.452(0.0663-1.0619)	Z=1.91, P=0.057

Table 2 - Levels of heavy metals in relation to lymph nodes involvement

	Positive LN (N = 24)	Negative LN (N = 21)	Significance test
Zn	0.218(0.066-0.917)	0.412(0.021-0.915)	Z=1.81, P=0.07
Pb	0.7315(0.0965-2.186)	1.632(0.845-2.98)	Z=4.35, P≤0.001
Co	4.5235(0.0-12.594)	4.134(0.224-14.1)	Z=0.73, P=0.47
Cd	0.1231(0.0-0.347)	0.333(0.0-0.951)	Z=4.11, P≤0.001
Fe	1.8238(0.0662-3.31)	2.21(0.159-5.774)	Z=1.82, P=0.07
Cr	0.452(0.009-1.366)	0.9843(0.0-2.03)	Z=2.69, P=0.007

On the other hand, the median value of serum level of Co was lower in patients with cholangiocarcinoma having negative LN involvement than in cholangiocarcinoma patients having positive LN involvement but the difference in the serum level between both groups was statistically insignificant.

Relationship to Tumor Differentiation

Table 3 shows that the median values of serum levels of Zn, Pb, and Cd which showed progressive increase in the serum levels of heavy metals from well differentiated to moderately differentiated to undifferentiated tumors, but this increase was statistically significant only in serum level of Pb.

Relationship to Tumor Markers and Survival

Table 4 shows that among the six heavy metals, only Cd showed a statistically significant correlation between its serum level and CA19.9 where the correlation is

positive while the correlation between its serum level and survival is negative.

DISCUSSION

In Egypt the number of newly diagnosed cases of cholangiocarcinoma increases annually, commonly occurring in male farmers and in rural residents (13). Closed Manzallah Lake in Dakahlia governorate is contaminated with heavy metals including cadmium, due to increased discharge of untreated industrial wastes and agricultural irrigation by wastewater (14). Hassanien assessed human health risk estimates in residents from different regions in Cairo, Egypt (15). He concluded that, the current levels of copper, chromium, cadmium, lead, manganese, vanadium, arsenic, nickel, antimony and titanium were higher than those considered safe for the general population. In Egypt, most of fish farms are depending on agriculture drainage water mixed with industrial, herbicides and

Table 3 - Levels of heavy metals in relation to tumor differentiation

	Tumor Differentiation			Significance test
	Well (N = 16)	Moderate (N = 23)	Undifferentiated (N = 6)	
Zn	0.2391(0.066-0.641)	0.345(0.021-0.917)	0.4478(0.255-0.915)	KW χ^2 =3.3, P=0.19
Pb	0.6515(0.0965-2.186)	1.377(0.361-2.98)	1.745(1.23-2.04)	KW χ^2 =14.62, P=0.001
Co	3.6555(0.224-9.45)	6.118(1.66-14.1)	3.555(1.89-8.037)	KW χ^2 =4.9, P=0.09
Cd	0.1231(0.0632-0.819)	0.237(0.0-0.819)	0.3015(0.079-0.613)	KW χ^2 =4.7, P=0.096
Fe	1.931(0.4751-3.31)	2.108(0.0662-5.774)	1.921(0.7872-3.844)	KW χ^2 =0.27, P=0.87
Cr	0.4772 (0.056-1.997)	0.83(0.0-1.987)	0.8095(0.2324-2.03)	KW χ^2 =1.1, P=0.58

Table 4 - Correlation pf heavy metals and tumor marker and survival

Metal		CA19-9	Survival
Zn	Correlation Coefficient	.045	.023
	Sig. (2-tailed)	.770	.882
Pb	Correlation Coefficient	.365*	-.287-
	Sig. (2-tailed)	.014	.056
Co	Correlation Coefficient	.118	.047
	Sig. (2-tailed)	.442	.757
Cd	Correlation Coefficient	.313*	-.361*
	Sig. (2-tailed)	.036	.015
Fe	Correlation Coefficient	.292	-.002-
	Sig. (2-tailed)	.052	.988
Cr	Correlation Coefficient	.205	-.177-
	Sig. (2-tailed)	.178	.245

the phosphate fertilizers which are considered the main source of cadmium in the environment.

A general feature of arsenic, cadmium, cobalt and nickel is the ability to enhance the mutagenicity and carcinogenicity of directly acting genotoxic agents by inhibiting the repair of damaged DNA. There is evidence that carcinogenic metal change the patterns of gene expression, either by activation of early genes (protooncogenes) or by interference with genes down-regulating cell growth (16).

In the current study there are statistically significant differences in the serum levels of Zn, Pb, Co, Cd and Fe between Patients having cholangiocarcinoma and control. Kriegel et al conducted a study to evaluate the association of pancreatic cancer patients in east Nile delta Egypt, in which serum cadmium level was significantly higher in water together with other heavy metals such as, lead, copper, nickel and chromium (17). These metals may be important environmental risk factors for incidence of other gastrointestinal cancers like esophageal cancer (18-22).

Progressive increase in the median values of serum levels of Pb was found to be correlated to the less differentiation of the tumors. This may be explained by oxidative stress induced by heavy metals which do not only facilitate tumor initiation by mutagenesis but also deplete the activities of cellular antioxidant enzymes through the interactions with their thiol groups and dysregulate cell growth and proliferation, leading to tumor promotion (23).

Cadmium only has a positive correlation with CA19-9 and negative correlation with the survival of the patients. Multiple studies have shown that while elevations in serum CA 19-9 is useful in the diagnosis of adenocarcinoma of the upper gastrointestinal tract and in monitoring of colonic carcinoma, its greatest sensitivity is in the detection of pancreatic adenocarcinoma (24).

Carcinogenicity of cadmium is due to multiple factors as indirect induction of oxidative stress, inhibition of DNA repair and deregulation of cell proliferation. In addition, cadmium has a unique mechanism of

disruption of the cadherin mediated cell-cell adhesion system and cell-cell communication. Cadmium specifically displaces calcium from the protein E-cadherin and impair the cell-cell adhesion thereby deregulating cell proliferation (25).

There is very little literature about possible carcinogenic effect on pancreatobiliary malignancy. The strongest evidence of association between cadmium exposure and pancreatic cancer emerged from the East Nile Delta region of Egypt (12). The Nile delta region has very high levels of pollution and is therefore a best setting for studying interrelationship between environmental pollution and the process of carcinogenesis.

CONCLUSIONS

In conclusion, this pilot study has shown a statistically significant association between serum levels of Zn, Pb, Co, Cd and Fe in Patients having cholangiocarcinoma. Future studies should expand on this pilot investigation by studying a larger number of cholangiocarcinoma patients and by collecting extensive information on the lifetime occupational, residential, and environmental exposures and dietary influences in order to explain the role of heavy metals in cholangiocarcinoma etiology in this population. It will also be important to examine genetic susceptibility and markers of genetic damage caused by environmental exposures to clarify the role of such exposures in cholangiocarcinoma.

Conflicts of Interest

No conflicts of interest were declared.

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