Research Journal of Environmental Toxicology

ISSN 1819-3420

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Environmental Toxicology

ISSN 1819-3420
DOI: 10.3923/rjet.2017.55.61

Research Article
Hepatocellular Carcinoma in Populations Consuming Heavy Metals Contaminated Plants in North Delta, Egypt

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Abstract

Background: Liver cancer is a widespread malady in Northern parts of Egypt, in which industrial and municipal heavy metals pollutants contaminate both water and soil used for growing edible field and vegetable crops. **Materials and Methods:** Case-control studies were carried out in three locations in North Delta region (Dakahlia, Kafr El-Sheikh and Damietta governorates) where lead, cadmium, arsenic and mercury are common pollutants. Clinical examination of 143 HCC patients living in heavy metals-polluted areas and 171 healthy individuals living in relatively clean, non-polluted areas were carried out. The investigation was confirmed by fine needle aspiration cytology, histological examination and alpha-fetoprotein level analysis. Heavy metals assay in blood, plants, soil and water were carried out using the atomic absorption spectrophotometry technique and data were statistically analyzed. **Results:** Demographic and clinical data of patients with HCC show that levels of heavy metals under investigation (Pb, Cd, As and Hg) were significantly higher in the blood of HCC patients compared to control subjects. **Conclusion:** Results address a strong correlation between the occurrence of these heavy metals in blood of HCC patients and their levels in irrigation water, soil and edible plants.

Key words: HCC, heavy metals pollution, lead, cadmium, arsenic, mercury, polluted irrigation water, polluted soil, polluted edible plants

Received: November 19, 2016 Accepted: February 03, 2017 Published: March 15, 2017

Citation: Mohamed A. Elwakil, Ekbal M. Abo-Hashem, Yasser M. Shabana, Mohamed A. El-Metwally, Ghada El-Kannishy, Ali M. El-Adl, Rokiah Anwar, Eman Fawzy, Narmin Saied and Mustafa M. El-Zayat, 2017. Hepatocellular carcinoma in populations consuming heavy metals contaminated plants in North Delta, Egypt. Res. J. Environ. Toxicol., 11:55-61.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver cancer, the 4th most common cancer and the second cause of cancer mortality in both sexes in Egypt has been wide-spreading in the past 10 years[1-3].

In recent years, the relationship between the environmental pollution and cancer epidemiology has been extensively studied. Populations in areas with high exposure to organic or inorganic pollutants may suffer from cancer or non-cancer mortality[4].

Although heavy metals disturb fundamental cellular processes, their role in the onset and progression of tumors is still not confirmed. However, environmental metal carcinogenesis is considered a major public health problem[5-7].

Therefore, the present case-control study aims at identifying the impact of environmental heavy metals pollution including: Lead, cadmium, arsenic and mercury on the development of hepatocellular carcinoma in occupational populations where drastically polluted water is used for agricultural irrigation.

MATERIALS AND METHODS

This study was conducted on 143 primary hepatocellular carcinoma patients recruited from those attending the out-patients and in-patients clinics of the Internal Medicine Hospital, Mansoura University, Egypt. The patients represented three governorates of North Delta region (Dakahlia, Kafr El-Sheikh and Damietta), where industrial and municipal wastes disposal were observed. The diagnosis was based on clinical examination, positive imaging studies (ultrasonography, computed tomography, angiography) and confirmed by fine needle aspiration cytology, histological examination and alpha-fetoprotein concentration of at least 500 ng mL^{-1} if needed.

Cases were compared to 171 healthy individuals living in a relatively non-contaminated area at Behira governorate. The control subjects were proven healthy by clinical and laboratory tests.

For all subjects enrolled in the study, explanation of the procedures was fulfilled and informed consents were obtained. Approval by the Research Ethics Committee of the University was also obtained.

Blood sampling: Samples of 6 mL of venous blood were collected from each subject and deposited in EDTA metal-free vacutainer tubes and kept at -20°C to be analyzed for heavy metals concentrations using atomic absorption spectrophotometry technique.

Heavy metals assessment

In subject's blood: Ten milliliters of a mixture of analytical graded acids HNO_3:H_2SO_4:$HClO_4$ in the ratio 5:1:1 were added to 1 mL of blood sample in 100 mL beaker then digested at 180-190°C for 3-4 h. After cooling, the ash was dissolved in bidistilled-deionized water acidified with 3% nitric acid and completed to a final volume of 50 mL. Lead, cadmium, mercury and arsenic concentrations (mg L^{-1}) were estimated by Buck Scientific Accusys "214" atomic absorption spectrophotometer[8].

In soil, water and edible crops: Heavy metals assessments were performed on 573 samples of irrigation water, field soil and plants collected from 94 geographical sites representing the three selected locations (Dakahlia, Kafr El-Sheikh and Damietta governorates). The longitudes and latitudes were determined on site by the aid of Geographical Positioning System (GPS). Representative samples of irrigation water (1000 mL), soil (1 kg), plants (1 kg) and plant products (1 kg) were collected from the allocated points and stored in a refrigerator until processed for the determination of their contents of lead, cadmium, mercury and arsenic. All soil and plant samples were air dried for 3 days followed by oven drying at 70°C until a fixed weight was obtained in two consecutive times. The concentrations of the four heavy metals were estimated. The results were reported as mg kg^{-1} dry weight.

Statistical analysis: Samples were randomly collected from different locations. The statistical analysis of data was performed using SPSS program (Statistical Package for Social Science) (SPSS, Inc., Chicago, IL) version 20. Kolmogorov-Smirnov tests of significance assumed that the data have a normal distribution. Qualitative data were presented as frequency and percentage. Chi-square and Fisher exact tests were used to compare the group means. Quantitative data were presented as minimum, maximum and mean along with their standard deviation. For comparison between two group means, student t-test was used. For comparisons among more than two group means, LSD test at $p = 0.001$ was used. Deviations from Hardy and Winberg equilibrium expectations were determined by using the chi-squared test. Odds ratio and 95% confidence interval were calculated. Pearson correlation coefficients were used to determine the degree of association between heavy metals in blood and in water, soil and edible crop samples. Linear regression was performed to detect the amounts of heavy metals in blood relative to their presence in water, soil and edible crops. Tests of significant were performed at probability $p \leq 0.05$ and 95% confidence interval.

RESULTS AND DISCUSSION

Demographic and clinical data of patients with HCC are shown in Table 1. The levels of heavy metals under investigation (Pb, Cd, As and Hg) were significantly higher in the blood of HCC patients who live in the three governorates (Table 2). These levels exceeded the international permissible levels in human blood, which are 0.015, 0.005, 0.005 and 0.06 ppm for Pb, Cd, As and Hg, respectively.

The probability levels of significance indicated that the difference among the concentrations of any given heavy metal is highly significant when compared against the control in each governorate.

Assessment of these heavy metals in the irrigation water, soil and edible plants grown in the contaminated areas in which HCC patients live, showed that they contain high concentrations of these heavy metals.

However, when perusal these data presented in Table 3-6 of the 4 tested heavy metals concentration, it was observed that these heavy metals were significantly higher in both water used for irrigation in the 3 governorates and in the soil irrigated with such polluted water as well as in the edible plant parts.

The differences in lead concentration in plants in the three governorates were not significant (<3 ppm). However, these differences would be due to the variable amounts of this heavy metal in Damietta if compared with its concentration in both governorates of Dakahlia and Kafr El-Sheikh.

These results are supported by Esposti *et al.*[9] who stated that HCC is probably induced by various environmental pollutants including classes of chemicals such as heavy metals. Being the first line of defence and detoxification, the liver is the main organ attacked by industrial chemicals polluting water and food products[10].

Survey presented here show that HCC is widely spread in the Northern region of Nile Delta where the municipal and industrial pollutants far exceed the permissible levels. Considering this fact and the impact of HCC on the community, this study was designed to study the possible correlation between the incidence of HCC and environmental heavy metal pollution in polluted areas. To the best of our knowledge, this is the 1st study to address this association in Egypt, where HCC incidence has risen to threatening levels.

In the present study, the association between the levels of 4 toxic heavy metals in human blood and the development of hepatocellular carcinoma in Northern Egypt is insured by estimates of correlation coefficient. Our results revealed significant differences between the levels of lead, mercury cadmium and arsenic in the blood of HCC patients and those

Table 1: Demographic and clinical data of hepatocellular carcinoma patients

Parameters	HCC (n = 143)
Age (years), Mean±SD	43.9±11.6
Gender, N (%)	
Males	51.5
Females	48.5
Tumor size, N (%)	
<3 cm	54.4
>3 cm	45.6
Multiplicity, N (%)	
Single	53.2
Multiple	46.8

Table 2: Heavy metals concentration in HCC patient's blood and control subjects' blood in the three governorates under investigation; Dakahlia, Kafr El-Sheikh and Damietta

Heavy metals concentration (ppm)	Governorates	Control (Mean±SD)	HCC patients (Mean±SD)	p-value
Lead	Behira (Check)	0.237±0.114	0.608±0.309	<0.001
	Dakahlia	-	0.593±0.253	<0.001
	Kafr El-Sheikh	-	0.783±0.396	<0.001
	Damietta	-	0.541±0.412	<0.001
Cadmium	Behira (Check)	0.006±0.002	0.038±0.014	<0.001
	Dakahlia	-	0.038±0.019	<0.001
	Kafr El-Sheikh	-	0.030±0.014	<0.001
	Damietta	-	0.047±0.021	<0.001
Arsenic	Behira (Check)	0.019±0.008	0.237±0.117	<0.001
	Dakahlia	-	0.237±0.117	<0.001
	Kafr El-Sheikh	-	0.148±0.072	<0.001
	Damietta	-	0.321±0.152	<0.001
Mercury	Behira (Check)	0.017±0.008	0.129±0.084	<0.001
	Dakahlia	-	0.109±0.056	<0.001
	Kafr El-Sheikh	-	0.089±0.044	<0.001
	Damietta	-	0.292±0.141	<0.001

p-value the probability of significant

Table 3: Lead concentrations (ppm) in water, Edible Plants (EP) and soil samples collected from three governorates in Egypt

	Dakahleia			Kafr El-Sheikh			Damietta			Control		
Lead	Water (N = 24)	EP (N = 112)	Soil (N = 23)	Water (N = 25)	EP (N = 127)	Soil (N = 19)	Water (N = 29)	EP (N = 59)	Soil (N = 27)	Water (N = 5)	EP (N = 6)	Soil (N = 5)
Mean±SD	0.248±0.11	24.4±15	342.8±228	0.253±0.126	24.5±17.5	332.5±188.9	0.222±0.145	27.1±20.2	268.2±180.1	0.024±0.016	8.3±3.1	40.6±14.1
Range												
Minimum	0.086	1.65	91.5	0.140	0.50	85.90	0.040	3.25	48.41	0.007	3.60	23.5
Maximum	0.532	68.5	888	0.635	76.5	798.3	0.570	67.2	731.3	0.069	12.2	55.2
Median	0.446	66.9	796.5	0.495	76.0	712.4	0.534	63.9	682.8	0.062	8.60	31.7
p-value	0.001	0.001	0.006	<0.001	<0.001	0.008	0.002	0.002	0.033			

Table 4: Cadmium concentrations (ppm) in water, Edible Plants (EP) and soil samples collected from three governorates in Egypt

	Dakahleia			Kafr El-Sheikh			Damietta			Control		
Lead	Water (N = 24)	EP (N = 112)	Soil (N = 23)	Water (N = 25)	EP (N = 127)	Soil (N = 19)	Water (N = 29)	EP (N = 59)	Soil (N = 27)	Water (N = 5)	EP (N = 6)	Soil (N = 5)
Mean±SD	0.059±0.027	1.21±0.876	3.60±1.104	0.048±0.019	1.46±0.865	2.61±1.17	0.035±0.017	1.302±0.892	2.16±1.353	0.015±0.01	0.17±0.076	0.467±0.274
Range												
Minimum	0.006	0.05	0.196	0.01	0.05	0.3	0.004	0.05	0.06	0.003	0.090	0.148
Maximum	0.167	4.80	14.8	0.204	4.75	10.02	0.570	4.725	7	0.044	0.300	0.750
Median	0.161	4.75	14.6	0.194	4.70	9.72	0.566	4.675	6.94	0.041	0.210	0.602
p-value	0.191	0.001	0.191	0.416	<0.001	0.416	0.539	0.001	0.539			

Table 5: Arsenic concentrations (ppm) in water, Edible Plants (EP) and soil samples collected from three governorates in Egypt

	Dakahleia			Kafr El-Sheikh			Damietta			Control		
Arsenic	Water (N = 24)	EP (N = 112)	Soil (N = 23)	Water (N = 25)	EP (N = 127)	Water (N = 24)	EP (N = 112)	Soil (N = 23)	Water (N = 25)	Water (N = 5)	Water (N = 24)	EP (N = 112)
Mean±SD	0.222±0.088	0.108±0.092	11.569±8.687	0.167±0.073	0.187±0.141	19.187±9.748	0.168±0.109	0.131±0.117	13.95±9.28	0.005±0.002	0.010±0.002	0.661±0.22
Range												
Minimum	0.065	0.009	0.910	0.007	0.006	1.870	0.002	0.004	0.420	0.002	0.008	0.365
Maximum	0.432	0.475	27.590	0.267	0.85	35.150	0.387	0.730	33.120	0.008	0.014	0.884
Median	0.367	0.466	26.68	0.26	0.844	33.28	0.385	0.726	32.700	0.006	0.006	0.519
p-value	<0.001	0.118	0.024	<0.001	0.003	<0.001	<0.001	0.066	0.003			

Table 6: Mercury concentrations (ppm) in water, Edible Plants (EP) and soil samples collected from three governorates in Egypt

	Dakahleia			Kafr El-Sheikh			Damietta			Control		
Mercury	Water (N = 24)	EP (N = 112)	Soil (N = 23)	Water (N = 25)	EP (N = 127)	Soil (N = 19)	Water (N = 29)	EP (N = 59)	Soil (N = 27)	Water (N = 5)	EP (N = 6)	Soil (N = 5)
Mean±SD	0.222±0.088	0.108±0.092	11.57±8.687	0.167±0.073	0.187±0.091	19.187±9.748	0.168±0.109	0.131±0.107	13.95±9.28	0.003±0.0008	0.322±0.112	0.373±0.067
Range												
Minimum	0.065	0.009	0.910	0.007	0.006	1.870	0.002	0.004	0.420	0.002	0.15	0.28
Maximum	0.432	0.475	27.59	0.267	0.85	35.15	0.387	0.730	33.12	0.004	0.44	0.44
Median	0.367	0.466	26.68	0.260	0.844	33.28	0.385	0.726	32.70	0.002	0.29	0.16
p-value	0.093	0.270	0.010	0.059	0.1	0.001	0.261	0.685	0.082			

levels in the control subjects in the three selected governorates (Table 2-6). These results provided evidence of strong association between each heavy metal tested and the development of HCC in patients living in areas or consuming food products polluted with these heavy metals.

The present study revealed that lead level in blood of the control subjects (237±114 μg L^{-1}) is still higher than the reference levels stated by Sponder *et al.*[11] as 70 and 90 μg L^{-1} for women and men, respectively. The HCC patient's blood levels showed significantly higher levels of lead (608±309 μg L^{-1}).

Water samples collected from the control area (GPS: N: 30° 44'56.7"- EO: 30°40'11.5") revealed lead concentration of 24±16 μg L^{-1}. This is in line with water lead levels obtained in a study conducted in Ontario, Canada, which showed that the average concentration of lead in water was in the range[12] of 1.1–30.7 μg L^{-1}.

Lead contaminated soil can pose a risk through direct uptake in the edible parts of field crops and vegetables. Control samples analyzed in the present study showed lead levels of 40.6±16.1 ppm, which lie within the range of the American Academy of Paediatrics (AAP). The non-contaminated soil contains lead concentration <50 ppm, however, soil lead levels in many urban areas exceed 200 ppm[13].

Samples of edible crops grown in the low-contaminated areas in the present study showed lead content of 8.3±3.1 ppm. United States Food and Drug Administration (FDA) has set a level of 0.5 μg mL^{-1} (= 0.5 ppm) for lead in products intended for use by infants and children and has banned the use of lead soldered food cans[14-16].

Cadmium exerts its toxic effects on several organs, including the liver and it is classified as a human carcinogen by the IARC[17]. It acts through diverse indirect mechanisms and has limited direct genotoxic activity[7].

The results presented in this study revealed that cadmium levels in blood of the control subjects was 6±2 μg L^{-1}, which is higher than the Human Biological Material (HBM)-II levels (4 μg L^{-1}) and far beyond the reference level (0.8 μg L^{-1}) according to Sponder *et al.*[11]. Blood samples drawn from HCC patients showed significantly higher levels of cadmium (38±14 μg L^{-1}). High levels of cadmium in blood is associated with liver morbidity, which is concordant with Hyder *et al.*[18], who stated that exposure to environmental pollution of cadmium was associated with hepatic necroinflammation. Cadmium exposure affects the liver causing hepatocyte swelling, fatty changes, focal necrosis, hepatocyte degeneration and abnormalities in biomarkers of liver function[19].

In water and soil samples collected in the present study, there were non-significant differences of cadmium levels between the HCC-infested governorates and the control area. However, a higher concentration of cadmium in edible crops was recorded in HCC-infested governorates in comparison with the control area (Table 4).

A risk for cancer-death may occur due to arsenic effects on the normal epigenetic transcriptional regulation[20], while millions of people is exposed to such toxic element, which persist in the environment. In the present study, high levels of arsenic have been recorded in blood samples of the control subjects (19±8 μg L^{-1}), which is far beyond the normal human level (1 μg L^{-1}) as stated by ATSDR[21]. Blood samples drawn from HCC patients revealed seriously higher concentrations of arsenic (148-321 μg L^{-1}) (Table 2).

Samples of soil collected in the control area revealed arsenic concentration of 0.661±0.220 mg kg^{-1}, which was significantly lower than it of those collected from localities in which patients with HCC are living.

The United States Environmental Protection Agency (EPA)[22] superfund risk model that gives a value of 0.43 ppm total soil arsenic for a cancer risk of 1 in 106 for exposure by soil ingestion. This has created a regulation of soil arsenic by individual states as the average 5 ppm. State standards for remedial action vary tremendously, but many require this when soil arsenic is above the natural background, which is often <10 ppm. Background soil arsenic levels have been shown to vary with soil type. Soil arsenic standards in other countries (Canada, UK, Netherlands and Australia) are generally in the range of 10-20 ppm for agricultural use of soils[23]. It was also reported that the soil and herbage survey in UK found total arsenic concentrations in the range of 0.5-143 mg kg^{-1} dry weight for rural soils, with a mean[24] of 10.9 mg kg^{-1}.

Regarding water samples, this study revealed that water arsenic concentration in control areas was 5±2 μg L^{-1}, which is compatible with the standards of World Health Organization (WHO)[25] and the US Environmental Protection Agency[22], which have recommended a threshold of 10 μg L^{-1} for inorganic arsenic concentration in drinking water. In the same context, ATSDR[21] stated that drinking water generally contains an average of 2 μg L^{-1} of arsenic, although higher levels have been measured in some parts of the US. The WHO has set 10 ppb (0.01 ppm) as the allowable level for arsenic in drinking water[25,22].

Present study revealed that arsenic concentrations in the edible crops collected in control areas was 0.010±0.002 mg kg^{-1} (= 0.01±0.002 ppm). Worthy to note that several countries, including the UK and Australia,

currently use a 1 ppm limit for arsenic in the edible crops and vegetables and this is often cited as a "Safe" level for rice[26].

Mercury as a transition metal promotes the formation of ROS, which enhances the peroxides and hydroxyl radicals leading to cell membrane damage and inhibits the activities of the free radical quenching enzymes[27].

This study revealed that the control blood mercury level was 17±8 μg L^{-1}, which is around the HBM-II levels (15 μg L^{-1}) but far beyond the reference level (2 μg L^{-1}) according to Sponder *et al.*[11]. However, blood samples drawn from HCC patients revealed seriously higher concentration of mercury (129±84 μg L^{-1}).

Soil samples collected from control areas showed significantly lower levels of mercury (0.373±0.067 ppm) compared to samples taken from other localities. Mercury level recorded in soil of the control area is well acceptable with the limit for mercury in soil set by Revis *et al.*[28] as 722 ppm, but considered far beyond the one recorded by a recent study, which reported that 95% of the organisms would be safe with a mercury concentration of 0.13 μg g^{-1} of soil (= 0.13 ppm)[29].

Water samples collected in the control areas recorded mercury level of 0.003±0.0008 mg L^{-1}. It is worth to mention that the maximum level of mercury accepted by the US EPA in 2008 was 0.002 mg L^{-1}.

CONCLUSION

The data presented in this study confirms a positive correlation between soil, irrigation water and edible plants contaminated with Pb, Cd, As and Hg and the incidence of HCC in people live in these polluted areas (Dakahlia, Kafr El-Sheikh and Damietta governorates).

SIGNIFICANCE STATEMENT

This study highlights the importance of using clean water for irrigating the edible crops as the results show a positive correlation between the incidence of hepatocellular carcinoma (HCC) among Egyptian farmers and other who live and feed on crops grown under stress of contaminated water with heavy metals. This evidence based work was recognized by the Egyptian Academy of Science and Technology and received the State of Merit Award in 2014. The tangible state of the art results presented here measure the accumulation of the heavy metals in blood of subjects as well as soil, water and edible plants irrigated with water contaminated with industrial and municipal effluents, which is known to be happened in 3rd world countries leading to liver cancer.

ACKNOWLEDGMENTS

The authors acknowledge Mansoura University for offering the facilities to carry out this research. This research was funded by Science and Technology Development Fund of Egypt (STDF).

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