Hepatocellular Carcinoma in Relation to Occupational and Environmental Heavy metals Exposure

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Abstract

Hepatitis C and B were considered the unique risk factors encountered for HCC. However epidemiological studies indicate that human liver is susceptible also to environmental carcinogens including heavy metals. In the current study lead and cadmium were examined to find the possible relation to either chronic hepatitis or hepatocellular carcinoma because those two heavy metals are widely distributed in the Egyptian community especially in rural community. Variety of pesticides, fertilizers in addition to drinking water contamination together with smoking were considered sources of different chemicals especially those two heavy metals. **Objectives**: To determine the possible relation between blood lead and cadmium and HCC and chronic hepatitis. **Methodology**: Three groups were included 50 HCC, 50 chronic hepatitis were compared to 50 matched control group. All subjects were asked to fill an interview questionnaire that covers detailed sociodemographic, occupational and environmental risk factors . Gas chromatography was used for measurement of blood heavy metals in whole blood sample. **Results**: blood cadmium level was higher among HCC group compared to chronic hepatitis group and controls (1.6$\pm$0.4, 1.2$\pm$0.3 and 1$\pm$0.4) respectively with a statistically significant difference (p0.01). No statistically significant difference has been the studied groups as regard blood lead level (14.8$\pm$5, 12.5$\pm$3.4 and 12$\pm$4.7 respectively p =0.21NS). ALT and AST were higher among HCC and chronic hepatitis group compared to controls (96$\pm$43, 76$\pm$55 and 23$\pm$5) and (78+40, 70+50.7 and 16.8+5 respectively p<0.001HS). Cadmium level was inversely correlated with albumin level (r=-0.30 and p=0.02). Cadmium is positively correlated versus age r=0.32 and p=0.02S. On the other hand no statistically significant correlation could be detected between cadmium with. Lead was positively correlated versus age (r=-0.29 and p=0.04S). On the other hand no statistically significant correlation could be detected between lead and other variables (p>0.05). No statistically significant difference between males and females as regard lead and cadmium. **Conclusion**: This study reinforces that blood cadmium may play a role in HCC but direct effect couldn't be confirmed. Smoking represents the main environmental source of cadmium among HCC patients. Further studies are needed to clarify direct effects of environmental risk factors in the pathogenesis and progression of liver cancer.

Key words: Cadmium and lead level, HCC, heavy metals and cancer liver

Introduction

Hepatocelluar carcinoma was considered one of the most fatal cancers with a life span ranged from 6 months to 1 year ranking it to be the fourth

highest cause of cancer-related deaths [1, 2], Hepatocellular carcinoma (HCC) has became the third most common malignancy worldwide, majority of deaths were in the developing countries [3]. Liver cancer especially hepatocellular carcinoma were on the top 4 causes of death [4]. Hepatitis B responsible for 66% of all cases of HCC, while HCV 42%), assuming that the relative risk of disease in both carriers is 20 [5]. Egypt has the highest prevalence of HCV in the world, ranging from 6 to 28%, with an average of approximately 13.8% in the general population and there is an expected increase in hepatitis C-related mortality in that country. Overall age adjusted incidence of HCC in Egypt was 10/100.000 according to Tanta cancer registry institute 2003[6]

Occupational liver cancer among workers of different categories of occupation with different types of exposures that include; farming, textile industry, petroleum-producing industry, metal industry and restaurant business has been examined in different literatures [11,18,22]. Different work place and environmental pollutants as hydrocarbons, pesticides, dyes in addition to heavy metals has been tested in different researches [21, 23,25]. None of these occupational factors has been studied in relation to the risk of liver cancer in Egypt. Few studies in Egypt examine the relation between HCC and aflatoxines, Single center study by Abdelrahman Ziady that found a significant association between aflatoxine and possibility of HCC. Other risk factors were described also to have an important impact like alcohol consumption, hemochromatosis in addition to heavy metals, and organic solvents [8, 16,20] . Low blood cadmium level may be associated with chronic renal and liver disease above 1mcg/L blood although this study cant confirm a strong direct relation between cadmium level and chronic liver disease but it spotlight the possibility of the additive effect of heavy metals with other risk factors [20].

Over decades due to rising prevalence of HCC many studies in Egypt were designed to find a possible relation between viral hepatitis B&C versus HCC [14, 16, 26, , 27]. Routes of transmission of HCV and B in addition to anti-shistozoma therapy have been examined [28, 29]. Some investigations aimed at determining aflatoxin levels in food items in Egypt produced conflicting results [16, 30, 31] Recent population-based study in Delta region has suggested that environmental factors might contribute to the incidence of HCC in Egypt including organic solvents from pesticides and heavy metals [7, 9,10]. According to local studies; Lower Egypt had a rising prevalence of HCC with a possibility of new risk factors in addition to HBV and HCV. Egypt is considered highly polluted country by heavy metals especially lead and cadmium from water, food and smoking in addition to different occupational exposures [6]. This study will explore the possible relation between these environmental pollutants and HCC.

Objectives

-To determine the possible relation between blood level of heavy metals (lead and cadmium) and HCC

-To find out epidemiological, environmental and occupational characteristics of HCC

Methodology

Type of the study: Case control study, three groups were enrolled in this study according to the following data. Power of 80% and confidence level of 95% with accepted margin of error of 5%. The manual equation used for sample size calculation takes into consideration highest possible prevalence of HCC among HCV cases .Total sample per each group were 50 subject totally 150 subject for the 3 groups (HCC with HCV infection , chronic HCV infection and healthy group as a controls respectively)

All subjects were asked to fill an interview questionnaire that cover current job that they had engaged and time frames. Residential histories were collected.

Laboratory methodology: all candidates were subjected to blood samples using heparinized tubes and taking 3 cm whole blood was used for measurement of blood lead and cadmium levels. Atomic absorption chromatography device of Perkinelmer's auto system GC was used for analysis as follows.

Cadmium in blood and plasma measured by graphite furnace atomic absorption spectroscopy (GFAAS) facilitated by a wet ashing pretreatment of samples resulted in good accuracy and reproducibility. The sample detection limit using this method was 0.4 µg/L . This method was also precise and highly reproducible in determining Cadmium in whole blood matrix may also be modified with diammonium hydrogen phosphate [39]

Blood lead level was measured using the same device detailed above as follow:
GFAAS used an electrically heated graphite tube to vaporize and atomize the analyte at
temperatures up to 3000 °C prior to its detection. Sample volumes of 10–50 µl could be
analyzed. Because the entire sample was atomized within a small volume, a dense atom population was produced. This technique is therefore very sensitive. Methods have been developed that can measure lead concentrations down to below 0.1 µg/dl [6, 14]; however, in routine use, the limit of detection is in the order of 1–2 µg/dl. GFAAS is currently one of the most commonly used methods for determining lead concentrations in blood. GFAAS is subject to greater potential interference than FAAS. This potential for interference has been reduced by
improved instrumentation design and by the application of various matrix modifiers. GFAAS,
however, required trained laboratory personnel to be set up and operated accurately [39].

Quality assurance:
QA refers to all the steps had already taken in occupational health unite lab in Ain Shams University to assure that the laboratory results are reliable. It covered the utilization of scientifically and technically sound practices for laboratory investigations, including the selection, collection, storage and transport of specimens and the recording, reporting and interpretation of results. Trained staff was responsible of doing the quality control procedures and to document. Internal quality control, which is a set of procedures used by the

staff of a laboratory for continuously assessing results as they are produced in order to determine whether they are reliable enough to be released; in addition to regular calibration of the devices by contracted company (Perkinelmer's) .

Ethical considerations: verbal consent form all participants was performed in addition to confidentiality of information. **Statistical analysis:** Statistical analysis was performed using SPSS statistical package version 16 (to complete two-sided analyses of descriptive statistics, one way ANOVA test was used to compare more than two groups as regard quantitative variable. Kruskall Wallis test was used instead of ANOVA test in non parametric data. Unpaired t-test was used to compare two groups as regard quantitative variable. Spearman correlation coefficient test was used to rank variables positively or inversely, P value >0.05 no significance, P<0.05 significant and P<0.001 highly significant difference.

Results

Table (1) There is no statistically significant difference between the studied groups as regard age (58±7.4, 53.5±8 and 56.7±10) among the studied groups respectively p=0.22NS. No statistically significant difference between both groups as regard gender P>0.05. As regard work duration; no significant difference as could be detected and disease duration p= 0.14 and 0.28NS respectively. As regard type of work farmers were the most frequent group among HCC group (46%) compared to 28% among chronic hepatitis group and 20% among controls with statistically significant difference p=0.02S. Majority of candidates in the studied groups were resident outside Cairo (88%, 80% and 86%) respectively with no significant difference between the studied groups. As regard pesticides exposure there is a higher frequency of farm exposure during farming processes in 46% among HCC group , while home exposure were found among 60% of control group with statistically significant difference in between p=0.002s. As regard type of pesticides used organic phosphorous was more frequent among HCC and chronic hepatitis group (26% and 20% respectively compared to 10% among control group. On the other hand pyrethroides were more frequent among control group with statistically significant difference p=0.03S.

Positive past history of bilharziasis were found among 66%, 56% and 60% among the studied groups respectively with no significant difference in between p =0.25NS. No statistically significant difference between the studied groups as regard frequency of smoking (32%, 26% and 30%) among the studied groups respectively with no detected significant difference p=0.76NS. Table (2) shows that cadmium level was higher among HCC group compared to chronic hepatitis group and controls (1.6±0.4, 1.2±0.3 and 1±0.4) respectively with statistically significant difference in between p0.01S. No statistically significant difference between the studied groups as regard blood lead level (14.8±5, 12.5±3.4 and 12±4.7) respectively p =0.21NS. as regard ALT and AST were higher among HCC and chronic hepatitis group compared to controls (96+43, 76+55 and 23+5) and (78+40, 70+50.7 and 16.8+5) respectively p<0.001HS. AFP was

dramatically higher among HCC group compared to the other two groups (201346+35467, 34.6+11 and 8.2+3) respectively p =0.000HS. Total bilirubin level was higher among HCC group 1.5+0.7 compared to 1.3+0.2 among chronic hepatitis and to 0.8+0.4 among control group p=0.000HS. on the other hand albumin level in gm/dl was lower among HCC group 3.2±1.7 compared to 3.6±1.5 among chronic hepatitis and 4.6±1.1 among controls p =0.000HS. As regard INR it was higher among HCC group compared to the other two groups (1.5±0.5, 1.2±0.4 and 1.03±0.2) respectively p =0.0001HS. Table (3) this table shows that cadmium level inversely correlated versus albumin level r=-0.30 and p=0.02. Cadmium is positively correlated versus age r=0.32 and p=0.02S. On the other hand no statistically significant correlation could be detected between cadmium versus other variables p>-0.05.

Table (4) this table shows that lead level was positively correlated versus age r=-0.29 and p=0.04S. On the other hand no statistically significant correlation could be detected between lead versus other variables p>-0.05. Table (5) this table shows that no statistically significant difference could be detected between males and females as regard lead and cadmium among HCC group (13±5 , 12.2±6, 1.6+0.3 and 1.54+0.7) respectively p>0.05. no significant difference between males and females among chronic hepatitis group as regard lead (11.4+4 , 10.8+5) and cadmium (1.05+0.4, 1.05+0.4 and 1.1±0.3) respectively. As regard control group no statistically significant difference between males versus females as regard lead (11.9±5, 12±4.3 and 1±0.3 and 1.04±0.4) p value >0.05. Table (6) shows that smoker group had higher blood cadmium level compared to non smokers (1.62+0.4 and 1.13+0.5) with statistical significant difference p =0.002S.

Discussion

Hepatocellular carcinoma in Egypt had represented a rising problem and hepatitis (HBV&HCV) was still the most confirmed risk factors but mandatory to search for other possible risk factors which may had additive or synergistic effects. The current study main question was if cadmium and lead had a role in hepatocellular carcinoma or chronic hepatitis. Total number of 150 candidates with average age respectively (58±7.4, 53.5±8 and 56.7±10) among the studied groups with no significant difference in between. As regard gender there was no statistically significant difference between the studied groups. Farmers were the most frequent occupation among HCC group with significant difference in comparison to the other groups. These results partially in agreement with a study by K. Heinemann which studied the association between occupation and liver cancer among women which included 317 HCC compared to 1789 controls. The study of K. Heinemann had concluded that chemical in workplace include heavy metals and solvents may play a synergistic or additive effect as a risk factors for HCC [32]. Farmers exposed in Egypt to a list of toxic heavy metals and solvents through daily exposure to pesticides and fertilizers in addition to high level of water and food contamination by these chemicals. As regard detailed residence of cases versus controls no statistically significant

difference were detected between the studied groups p>0.05. The current study shows that pesticides commonly used in farming (eg. malathion and methyl bromide) which were still a common names known by public was more frequent among both groups HCC and chronic hepatitis with significant difference in comparison to control group p<0.05. These findings were in agreement with as a study by Benhua Zhao et al which concluded significant relation between organochlorine and organic phosphorous pesticides and HCC in a group of exposed workers in China [40]. Pesticides may affect the liver by more than mechanism either direct toxicity of the active ingredients , or the solvents used which already well known hepatotoxic in addition to synergistic effect with other risk factors especially viral hepatitis.

Although the current study can't directly connected between HCC and farming as an occupation but it strongly spotlighted the presence of other additive risk factors to the viral hepatitis. As regard residence majority of Eldemrdash patients already referred from outside Cairo due to deficiency of appropriate care in frontier areas. But still it reinforced the possibility of association between nature of these communities in Egypt and presence of high chemical pollution especially nowadays. Taking into consideration the inevitable confounding factors for HCC as HCV infection which were already more frequent in these governorates especially Kafr Elsheekh , Behira , Gharbiah , Beni Swif and Menia (37) . Except smoking effect; epidemiological characteristics in the current study in agreement with a study by Amr S 2010 which include 150 cases of HCC compared to 150 controls this study concluded that occupational exposure may play an important role in the development of HCC. Farming, industrial exposures and cigarette smoking may increase the risk of HCC among HCV-seropositive individuals. Future research focusing on mechanisms of occupational exposures among HCV patients in this population was needed. On the other hand a smoker group with HCC had higher cadmium compared to non smokers although no significant difference between the studied groups as regard frequency of smoking but this explained that smoking was considered the main environmental source of cadmium among HCC according to the current study (33).

In the current study although blood cadmium was not the ideal marker for chronic exposure but also can't exclude hepatorenal syndrome or GFR declined by different degrees among the studied groups of HCC or chronic hepatitis. Urinary cadmium in those cases with liver cirrhosis years ago may not accurately represent their body burden.

As regard blood cadmium and blood lead level; the current study concluded that HCC group had higher blood cadmium level compared to controls with statistically significant difference. These results agree with Amr's study described above which detect high level of cadmium among HCC patients in addition to lead. Confirmation that cadmium level increased among HCC group was still may be affected by metallothionin level which was already declined among HCC patients and this may increase the free blood cadmium but these findings couldn't confirmed in any study.

Blood lead level in the current study not significantly different between the

studied groups. These results were in agreement with another study by Fujita et al 2006 which concluded that smoking play a strong additive effect together with HCV in pathogenesis of HCC odd's ratio (9.6) and this support the effect of cadmium as cigarette smoking is considered the one of the most important sources of environment cadmium but this study can't confirm significant relation between BLL versus HCC [34]. Although the OSHA trigger level of blood cadmium is 5µg/L which require medical surveillance for exposed workers but nearly all the above studies in addition to many recent researches relays on low level long term exposure to environmental cadmium and cancer liver which may additively affect other risk factors (35).

Cadmium is a heavy metal of considerable environmental and occupational concern. It's classified as human carcinogens by several regulatory agencies. The most convincing data that comes from studies on occupational lung cancer. Cadmium exposure has also been linked to human prostate and renal cancer, although this linkage is weaker than for lung cancer. Other target sites of cadmium carcinogenesis in humans, such as liver, pancreas and stomach, are considered equivocal. In animals, cadmium effectively induces cancers at multiple sites and by various routes. The most confirmed mechanism of Cd carcinogenesis was aberrant gene expression, inhibition of DNA damage repair, induction of oxidative stress, and inhibition of apoptosis. The available evidence indicates that, perhaps, oxidative stress plays a central role in Cd carcinogenesis because of its involvement in Cd-induced aberrant gene expression, inhibition of DNA damage repair, and apoptosis (38). As regard lead although no significant relation could be detected in the current study to HCC but still high level of pollution of lead of different sources in Egypt especially in Lower Egypt governorates mainly from water may play a role in oxidative stress to different organs include live and this may facilitate the effect of other stressors.

The current study concluded that cadmium inversely correlated versus serum albumin r=-0.30 and p=0.02. Cadmium is positively correlated versus age r=0.32 and p=0.02S. On the other hand no statistically significant correlation could be detected between cadmium versus other variables p>0.05. These results were in agreement with a study by Lovásová E et al 2013 which concluded significant inverse correlation between blood cadmium and serum albumin and positive correlation versus liver enzymes in rates. This may reflect the possible effect of chronic cadmium exposure on liver function and hepatocytes through oxidative stress mechanism [36].

As regard the positive correlation detected between lead and cadmium versus age in the current study may explained due to cumulative character of these heavy metals with age and this agree with a study by Fujita which conclude the same results. Long term exposure to cadmium had direct toxic effect on hepatocytes but this effect still not confirmed on human. As regard gender in the current study effect no statistically significant difference were detected between males and females as regard cadmium or lead p>0.05. **Conclusion**: this study reinforce that blood cadmium may play a role in HCC

but direct effect couldn't confirmed. Smoking represents the main environmental source of cadmium among HCC patients. Further studies are needed to clarify direct effects of environmental risk factors in the pathogenesis and progression of liver cancer.

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Table (1) Comparison between the studied groups as regard sociodemographic data

Variables	**HCC** N=50	**Chronic hepatitis** N=50	**Controls** N=50	P
Age (mean±SD)	58±7.4	53.5±8	56.7±10	0.22
Disease duration(yrs) (mean±SD)	5±4.6	3.5±2	-	0.14
Work duration(yrs) (mean±SD)	28±12	23.6±11	23.9±5	0.28
Gender: N(%)				0.32
Male	35(70%)	30(60%)	32(64%)	
Female	15(30%)	20(40%)	18(36%)	
Current Work: N(%)				**0.02***
Retired	10(20%)	8(16%)	4(12%)	
House wife	11(22%)	20(40%)	12(24%)	
Farmer	23(46%)	14(28%)	10(20%)	
Clark	3(6%)	5(10%)	15(30%)	
Manual work	3(6%)	3(6%)	9(18%)	
Residence : N(%)				0.35
Outside Cairo	44(88%)	40(80%)	43(86%)	
Cairo	6(12%)	10(20%)	7(14%)	
Residence details				0.21
Near to factories	15(30%)	7(14%)	10(20%)	
Near to waste collection area	8(16%)	9(18%)	8(16%)	
Rural area away from factories	15(30%)	20(40%)	14(28%)	
Old house >20yrs	12(24%)	14(28%)	18(36%)	
Pesticides exposure				**0.002***
In farming	23(46%)	11(22%)	5(10%)	**S**
Home	20(40%)	25(50%)	30(60%)	
Workplace	7(14%)	8(16%)	10(20%)	
others	0	6(12%)	5(10%)	
Types of pesticides				**0.03***
Organic phosphorus	13(26%)	10(20%)	5(10%)	**S**
(malathion)	10(20%)	15(30%)	35(70%)	
Pyrethroides	20(40%)	15(30%)	5(10%)	
Rodenticides	7(14%)	10(20%)	5(10%)	
Others				
Sources of drinking water				0.15
Municipal	20 (40%)	28(56%)	25(50%)	
Underground	17(34%)	10(20%)	11(22%)	
Portable **(**purchased per day)	13(26%)	12(24%)	14(28%)	
Smoking	16(32%)	13(26%)	15(30%)	0.76
Past history of bilharziasis	33(66%)	28(56%)	30(60%)	0.25

Table (2) Comparison between the studied groups as regard liver profile, BLL and blood cadmium level

Variables	**HCC** N=50	**Chronic hepatitis** N=50	**Controls** N=50	P	LSD(least significant difference)post hoc test
ALT(IU/dl)	96±43	76±55	23±5	**0.000HS#**	
AST(IU/dl)	78±40	70±50.7	16.8±5	**0.000HS#**	
AFP(mg/dl**)**	201346±35467	34.6±11	8.2±3	**0.000HS#**	
INR	1.5±0.5	1.2±0.4	1.03±0.2	**0.000HS** **<0.001***	HCC and chr. Hep. versus controls =0.000HS
Total Bilirubin (mg/dl)	1.5±0.7	1.3±0.2	0.8±0.4	**0.000HS**	HCC and chr. Hep. versus controls =0.000HS
Albumin (gm/dl**)**	3.2±1.7	3.6±1.5	4.6±1.1	**0.000HS**	HCC and chr. Hep. versus controls =0.000HS
Lead (µg/dl**)**	14.8±5	12.5±3.4	12±4.7	0.21	
Cadmium (µg/L)	1.6±0.4	1.2+0.3	1±0.4	**0.01***	-HCC versus controls =0.03HS

P<0.05 significant P<0.001highly significant P>0.05 non significant
 # Kruskall Wallis test

Table (3) Correlation between blood cadmium level versus different laboratory data among the studied groups

Variables	HCC		Chronic hepatitis		Controls	
	r	P	r	P	r	P
ALT(IU/dl)	0.09	0.53	0.13	0.33	-0.08	0.68
AST(IU/dl)	0.11	0.47	-0.08	0.65	0.21	0.13
AFP(mg/dl**)**	0.05	0.76	0.14	0.34	0.15	0.30
INR	-0.03	0.80	0.25	0.48	0.19	0.28
Total Bilirubin (mg/dl)	0.14	0.35	0.15	0.50	0.16	0.23
Albumin (gm/dl**)**	**-0.30**	**0.03***	-0.13	0.37	0.11	0.30
Lead (µg/dl**)**	0.08	0.13	0.22	0.12	0.17	0.31
Age	**0.32***	**0.02***	0.11	0.40	0.20	0.21
Work duration	0.12	0.40	0.15	0.23	0.06	0.78
Disease duration	0.09	0.60	0.10	0.28	0.22	0.16
Number of focal lesions	0.07	0.68	0.11	0.20	0.17	0.28

***P<0.05 significant **P<0.001highly significant no stars P>0.05 non significant**

Table (4) Correlation between blood lead level versus different parameters among the studied groups

Variables	HCC		Chronic hepatitis		Controls	
	r	P	r	P	r	P
ALT(IU/dl)	0.13	0.23	0.16	0.27	0.11	0.37
AST(IU/dl)	0.19	0.27	-0.18	0.25	0.20	0.16
AFP(mg/dl**)**	-0.13	0.46	-0.14	0.34	0.13	0.20
INR	0.22	0.13	0.25	0.48	-0.19	0.38
Total Bilirubin (mg/dl)	0.24	0.15	0.15	0.50	0.07	0.83
Albumin (gm/dl**)**	-0.20	0.12	-0.13	0.37	0.17	0.26
Lead (µg/dl**)**	0.03	0.56	0.22	0.12	0.12	0.34
Age	**0.29**	**0.04***	**0.31**	**0.03***	0.21	0.10
Work duration	0.11	0.38	0.10	0.26	0.14	0.38
Disease duration	0.12	0.40	0.13	0.22	0.08	0.76
Number of focal lesions	0.23	0.40	0.24	0.15	0.03	0.88

***P<0.05 significant **P<0.001highly significant no stars P>0.05 non significant**

Table (5) Comparison between males and females as regard lead and cadmium level among the studied groups

Variables	HCC		Chronic hepatitis		Controls	
	Male	Female	Male	Female	Male	Female
Lead	13±5	12.2±6	11.4±4	10.8±5	11.9±5	12±4.3
t	1.03		0.6		0.9	
P	0.33NS		0.78NS		0.84NS	
Cadmium	1.6±0.3	1.54±0.7	1.05±0.4	1.1±0.3	1±0.3	1.04±0.4
t	0.07		0.11		0.06	
P	0.94NS		0.65NS		0.89NS	

Table (6) Comparison between smoking versus cadmium level among HCC group

Variables	Smokers N=16	Non smokers N=34	P
Mean±SD	1.62±0.4	1.13±0.5	**0.002***