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BIOCHEMICAL AND ENVIRONMENTAL STUDIES ON HEPATOMA PATIENTS IN EGYPT

By

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دراسات كيموحيوية وبيئية تشمل مرضى سرطان الكبد في مصر

ناديه زخاري و عبد الباسط الأعصر و ميخائيل ميخائيل
سلوى الدمرداش و رفعت كامل و محمد حمزه

شملت هذه الدراسة ١٠٢ مريض مصابون بسرطان الكبد ومعظمهم من مرضى المعهد القومي للأورام – جامعة القاهرة في الفترة من ١٩٨٨ – ١٩٩١م .

تم أخذ بيانات شخصية وبيئية لكل مريض وتشمل سنه – جنسه – وظيفته – وما إذا كان قد سبق له العدوى بالبلهارسيا أم لا . كذلك أخذ في الاعتبار تعرضهم بصفة دورية لبعض الكيماويات المتمثلة في المبيدات الحشرية ومبيدات الفئران ومبيدات الحشائش وكذلك الأسمدة الكيميائية .

وقد أوضح البحث أن أغلب المرضى يقع سنهم مابين العقد الخامس (٣٢,٤٪) والعقد السادس (٢٨,٤٪) . وقد شخص ٨٩,٢ في المائة من هؤلاء المرضى على أن مرضهم من النوع المعروف بإسم السرطان الخلوي الكبدي . بينما ١٠,٨٪ منهم كان يعاني من أنواع أخرى من سرطانات الكبد . كذلك وجد أن ٤٣٪ من المرضى كانوا يعملون بالفلاحة وسبق لهم التعرض للمبيدات الحشرية المختلفة والأسمدة الكيميائية أثناء عملهم . كما وجد أن ٤١,٢٪ من المرضى قد سبق لهم الاصابة بمرض البلهارسيا .

أما بالنسبة للدراسات الكيموحيوية في هذا البحث ، فقد أوضحت النتائج ارتفاع ملحوظ في مصل مرضى سرطان الكبد لدليل الأورام الفا فيتوبروتين وكذلك مستوى نشاط كل من انزيم الفوسفاتيز القاعدي ، ٥ – نيو كليو تياديز ، GOT , GPT والجاما جلوتيمايل ترانسفاريز بالمقارنة إلى مستواهم في الأشخاص الأصحاء .

وبمقارنة المجموعة المصابة بسرطان الكبد من نوع السرطان الخلوي الكبدي والمجاميع المصابة بسرطانات الكبد الأخرى وجد أن أنزيم ٥ – نيوكليو تياديز كان مستوى نشاطه مرتفع كثيراً في مجموعة السرطان الخلوي الكبدي عنه في المجموعات الأخرى .

أما بالنسبة لدليل الأورام CEA فقد أسفر البحث عن عدم جدوى قياسه في مصل مرضى سرطان الكبد أيا كان نوعه .

وبقياس محتوى الجليكوجين هستوكيميائيا في نسيج الكبد المصاب بالسرطان وجد أن محتواه ضئيل في الخلايا المصابة بالسرطان بينما كان النسيج المدعم غني بالجليكوجين .

Key Words: Hepatoma-environmental factors, Bilharziasis, Liver enzymes

ABSTRACT

The present study was carried out on 102 hepatoma patients collected mainly from the National Cancer Institute, Cairo University, during the period 1988-1991 . Environmental and personal data were collected for each patient. These data included their age, sex and occupation. Previous history of fertilisers were also considered. The study revealed a peak of age of primary liver cancer, among Egyptians, between the fifth and sixth decades, since 32.4% and 28.4% of patients were aging from 50-60 and 60-70 years old, respectively. Results showed that 89.2% of the patients were diagnosed as hepatocellular carcinoma (HCC) whereas 10.8% of them suffered other types of hepatoma. 43 of the patients were farmers and exposed to chemical agents during their work. An intimate relation was observed between hepatoma patients and bilharziasis, which dominated in 41.2% of the patients investigated. The biochemical parameters investigated, revealed significant elevation in alpha fetoprotein level (AFP) in hepatoma patients as compared to controls, whereas the carcinoembryonic antigen (CEA) did not show any significant changes, regardless the histopathological type of hepatoma. Liver enzymes, namely 5-nucleotidase (5-Nase), leucine, aminopeptidase (LAP), transaminases (AST and ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT); revealed significant elevation in hepatoma patients as compared to their corresponding levels among controls. 5-Nase was significantly elevated in sera of HCC patients when compared to sera of patients suffering from other types of hepatoma. The periodic acid Schiff (PAS) technique was used to study the content of glycogen in liver tissue, histochemically. The results revealed loss of glycogen inside the hepatoma cells, whereas the stroma of the tumours were abundantly rich with glycogen.

INTRODUCTION

Primary malignant liver tumour represent a growing problem in developing countries. About 80-90% of these tumours are hepatocellular carcinoma (HCC)[1]. In some parts of Africa and Asia, HCC is the most common malignant tumour, with a population incidence ranging from 1.0 % in Mozambique and Taiwan, to 3.4% in Singapore.

In Egypt, the registry of National Cancer Institute[2] has shown that primary liver cancer represents 18.1% of digestive malignancies and 3.0% total malignancies. According to this registry, HCC showed a gradual annual increased rate from 54 cases, representing 1.93% total malignancies in 1985, to 2.95% total malignancies in 1989.

The most common environmental liver diseases encountered in Egypt are schistosomiasis, viral hepatitis, chemical intoxication, abuse of drugs and alcohols. These diseases represents an imbalance between man and his environment.

The present study was performed on primary liver cancer patients, aiming to study the ecological circumstances of these patients. The environmental studies were exemplified by age, sex, occupation, bilharziasis and exposure to chemicals. The present study was also aiming to pinpoint some biochemical and histochemical laboratory tests effectively aiding in the diagnosis of primary liver cancer.

MATERIALS AND METHODS

I. Environmental Studies

The present study was carried out on 102 hepatoma inpatients from the National Cancer Institute in Cairo (NCI). Their ages ranged from 7 months to 72 years. 80 of those patients were males and 22 were females.

The control group was represented by fifteen normal volunteers, 10 of those were males and 5 were females, whose ages ranged from 18 to 53 years. Environmental and personal data were collected for each individual case. These data were concerned with their age, sex, occupation, bilharzial history and long term therapy.

Exposure to chemical agents such as insecticides, pesticides, fertilisers or herbicides were also considered.

II Biochemical Studies

Serum was collected from 52 of these patients before receiving any chemotherapeutic, radiotherapeutic or surgical treatment. The serum was subjected to the following investigation:

1. Determination of Tumour Markers

Alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA) were analyzed using ELISA technique[3].

2. Serum Enzymatic Activity Levels

-5'-Nucleotidase (5'-Nase) was determined according to the method of El-Aaser and El-Merzabani[4].

-Leucine aminopeptidase (LAP) was determined according to the method of Nagel et al[5].

-Gamma glutamyl transferase (GGT) was determined according to the method of Szasz[6].

-Alkaline phosphatase (ALP) was determined according to the method of Ratliff and Hall[7].

-Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined according to the method of Reitman and Frankel[8].

III. Histochemcial Studies

The glycogen content of hepatoma tissue was determined histochemically using the periodic acid Schiff's reaction (PAS) according to the method of Tohamy et al[9].

IV. Statistical Analysis were performed using the hypothesis (t) test[10].

RESULTS

Patients were distributed according to different types of hepatomas and the results were represented in Table 1. HCC dominated over the other histopathological types, representing 89.2% of the cases investigated.

Table 2 represent the ages of patients investigated. The highest frequency was observed between 50 and 60 years old.

Table 3 represents the sex difference among hepatoma patients. Among the 91 HCC patients, 75 were males and 16 were females. In case of patients suffering other types of hepatomas, 5 were males and 6 were females.

Table 4 represents the distribution of hepatoma patients according to their occupation, bilharzial infestation, exposure to chemical agents as well as their long term therapy of diabetes or hypertension. Among the 91 HCC patients 43 were farmers and exposed to chemical agents, 4 patients were bilharzial and 9 were under treatment with drugs of either diabetes or hypertension. In case of the 11 patients suffering other types of hepatomas there were no farmers or individuals exposed to chemical agents, only 1 patient was bilharzial and 1 patient was under treatment with drugs of diabetes.

Table 5 represents the level of AFP and CEA in patients and control sera. AFP was significantly elevated among hepatoma patients (208.9 IU ± 197) as compared to controls (1.4 IU ± 1.0), ($P<.0001$). On the other hand no significant difference were observed concerning CEA levels. When HCC patients were compared with other types of hepatoma no significant changes were observed between the two groups with respect to AFP or CEA levels Table 6.

Table 7 revealed that liver enzymes activities were significantly elevated in sera of hepatoma patients as compared to their corresponding levels in the sera of controls.

Table 8 illustrated that 5-Nase was specifically markedly elevated in HCC patients (52.1 IU ± 24) when compared to other types of hepatomas (30.8 IU ± 13.2 and $P = 0.02$). On the other hand non significant changes were observed concerning the other investigated enzymes among the different types of hepatomas.

Histochemical studies revealed variable staining pattern for the PAS reaction. The same tissue section contained both hepatocytes devoid of glycogen and others containing glycogen. On the other hand the stroma of the malignant tissue was abundantly full of glycogen (Photo 1).

Table 1
Distribution of the patients according to different types of hepatomas

Diagnosis	Frequency	%
Hepatocellular Carcinoma	91	89.2
Cholangeo Carcinoma	4	3.9
Hepatoblastoma	4	3.9
Hemangiosarcoma	2	2.0
Carcinosarcoma	1	1.0
Total	102	100 %

Table 2
Distribution of the patients according to age

Age in years	Frequency	%
0-2	4	3.92
2-4	1	0.98
4-6	1	0.98
6-8	1	0.98
8-10	0	0.00
10-20	0	0.00
20-30	2	1.96
30-40	5	4.90
40-50	17	16.67
50-60	33	32.35
60-70	29	28.43
70-80	9	8.82
Total	102	100%

Table 3
Distribution of hepatoma patients according to sex.

Tumor Type	Total		Male		Female	
HCC	(91)	100	(75)[a]	82.4[b]	(16)	17.6
Other types of hepatoma	(11)	100	(5)	45.5	(6)	54.5
Total hepatoma patients	(102)	100	(80)	78.4	(22)	21.6
P-Value			0.02 Significant			

a: Results are represented as number of individuals
b: Results are represented as percent, taking total as 100 %.

Table 4
Distribution of hepatoma patients according to environmental factors (occupation, bilharzial infestation, chemical agents and long term therapy)

Tumour Type	Occupation		Bilharzial		Chemicals		Long term therapy.	
	Farmers	Non	+ve	-ve	+ve	-ve	+ve	-ve
HCC	43	48	41	50	43	48	9	82
Other Hepatoma	0	11	1	10	0	11	1	10
Total Hepatoma Patients	43	59	42	60	43	59	10	92
P-Value	0.007		0.05		0.007		0.93	

Table 5
Comparison of AFP and CEA in controls and hepatoma patients

Group	AFP	CEA
Control	1.4 ± 1	0.59 ± 0.47
Hepatoma patients	209 ± 197.7	3.3 ± 8.7
P-Value	<0.0001	0.117

- Results of AFP is represented as mean IU ± S. D. whereas CEA is represented as mean ng/ml ± S. D.
- Normal values: AFP up to 10 IU, CEA up to 3 ng/ml.

Table 6
AFP and CEA in HCC and other types of hepatomas

Tumour Type	AFP	CEA
HCC	224.3 ± 203.2	3.3 ± 3.7
Other types of hepatoma	91.7 ± 91.7	3.4 ± 2.9
P-Value	0.06	0.49

Legends asTable 5

Table 7
Liver enzymes in patients and controls

Enzyme	Hepatoma Patients	Control	P-Value
5-Nase	49.6 ± 23.9	9.6 ± 3.5	<0.0001
LAP	42.0 ± 22.2	14.5 ± 4.0	<0.0001
AST	44.8 ± 36.5	8.3 ± 2.2	<0.0001
ALT	37.5 ± 40.4	7.5 ± 1.6	<0.0001
ALP	262.6 ± 132.8	105.5 ± 16.1	<0.0001
GGT	88.0 ± 29.7	18.3 ± 4.3	<0.0001

Enzymatic activity levels are represented as mean ±S. D. in international units.
Normal values: 5-Nase (1-15 IU/ml); LAP (8-22 IU/L) AST and ALT (up to 12 IU/L) ALP (60-170 IU/L) GGT (4-28 IU/L).

Table 8
Liver enzymes in patients with HCC and other types of hepatomas.

Enzyme	HCC	Other Types of Hepatoma	P-Value
5-Nase	52.1 ± 24.0	30.8 ± 13.2	0.02
LAP	41.9 ± 22.4	42.3 ± 22.2	0.48
AST	47.2 ± 37.6	25.7 ± 20.1	0.09
ALT	39.2 ± 42.0	25.2 ± 24.1	0.22
ALP	267.0 ± 136.0	229.0 ± 107.0	0.26
GGT	89.6 ± 29.3	75.7 ± 32.2	0.14

Legends as Table 7

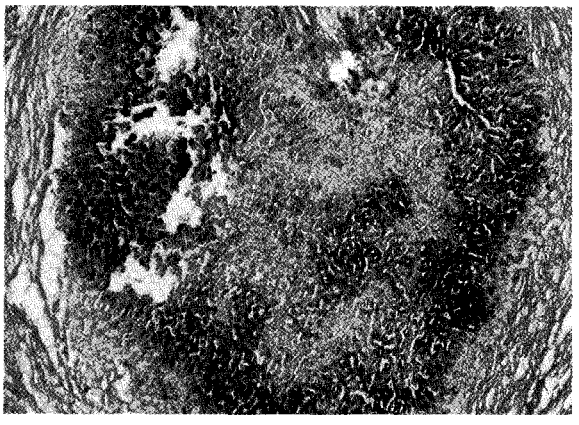

Photo (1): PAS reaction in liver tissue of hepatoma patient, demonstrating glycogen distribution among hepatic cells. Notice the accumulation of glycogen on the periphery of the tumor cellls, meanwhile, the cells at the center were devoid of glycogen, (X 100).

DISCUSSION

The present study revealed an increased incidence of HCC which comprised 89.2% of primary hepatomas, followed by hepatoblastoma and cholangiocarcinoma. These findings are in accordance with those obtained by Mokhtar[2] who reported that primary liver tumours were mostly HCC type.

In the present study only 7 patients were younger than 10 years, all of which were diagnosed as hepatoblastoma and these were the only hepatoblastoma patients in our study. This matches with the data of Farhi et al[11] who reported the hepatoma is a disease of adults, with rare incidence among children. Virtually all hepatoblastomas occurs before 5 years of age, with 65% occurring in children younger than 2 years old[12].

Although the peak of the unimodel age distribution of the hepatoma group of patients included in this study was in the 5th decade, Smalley et al[13] reported a different unimodel age distribution with a peak at the seventh decade, indicating a lower mean age for our population than for western communities. In this respect it is worthy to mention that Kew[14] reported an earlier peak age of HCC in high incidence areas of HCC and hepatitis B infection than in lower areas. On the other hand the age distribution of our cases were similar to those of the Japanese patients[15], whereas in South East Asia, which have the highest incidence of HCC, the peak of incidence was even in fourth decade[16].

In general, males are more prone to develop HCC than females. The estimated male female ratio was 3.6:1 in our present study whereas other ratios of 6:1 and 2.2:1 were reported by Wood et al[17] and Smalley et al.[13]; respectively. Anthony[18] reported that this ratio is higher in parts where the tumour is common. This might be explained by the fact that chronic hepatitis B viral infection is more common among males[19]. Recently, androgen receptors have been demonstrated in HCC cells, which might play a part in hepatic growth, regeneration and carcinogenesis[20].

Regarding the occupation of the patient investigated, the present study revealed that 42.2% of whom were farmers. No doubt that these farmers are occasionally exposed to different types of chemicals during their daily work. These chemicals included insecticides, pesticides, herbicides and fertilizers, which are quite established as carcinogenic agents[21]. On the other hand, these farmers were also affected with bilharziasis. The present study revealed a high incidence of bilharziasis among the hepatoma patients. The role of bilharziasis as aetiological factor for HCC may be indirect, as it might be a cofactor with hepatitis B viral infection. In this respect , Bassily et al;[22] demonstrated that hepatitis B and C viral infection were common among patients with hepatosplenic bilharziasis. Also Li[23] said that complicated hepatitis B viral infection may be one of the major causes of HCC development in patients with schistosomiasis Japonica.

Regarding the biochemical parameters investigated, it was found that all the liver enzymes were significantly elevated in hepatoma patients as compared to the control subjects, regardless the histopathological type of hepatoma, with the exception of 5-Nase, which was significantly markedly elevated in HCC patients when compared to other types of hepatoma patients ($P = 0.02$). This reveals that 5-Nase could be considered specific for HCC type of hepatoma. It is worth mentioning that it is more sensitive than ALP in cases of neoplastic liver diseases, when skeletal lesions are absent[24].

The present study revealed that serum levels of alpha fetoprotein (AFP) was normal in 12% of the patients (up to 10 IU/ml). 31% of the patients showed a serum level between 35-100 IU/ml, 15% were between 100 and 200 IU/ml, 35% were from 200 to 500 IU/ml, and 7% were over 500 IU/ml. Most of the patients which had AFP between 200 and 500 IU/ml were diagnosed as HCC grade II, and those over 500 IU/ml were diagnosed HCC grade III.

Nomura et al.[25] found a marked increase in the rate of a negative AFP (<20 ng/ml) in HCCs, with tumour diameter of 2 cm or less. They reported that the positive rate generally correlates with the tumour size. However, a wide variation AFP levels exists among the small groups and 6.4% of cases in the group of small size liver tumour (2 cm) had high AFP levels of 1000 to 5000 ng/ml. Accordingly, the degree of tumour differentiation appears to be more important than the tumour size in determining the level of AFP produced by HCCs. Small sized HCC patients with high levels of AFP had a low survival rate as compared with those having lower levels of AFP. They also report higher AFP level among HBsAg positive HCCs.

AFP levels in HCC patients with and without cirrhosis are not significantly different[26]. A fibrolamellar type of HCC shows low or negative AFP values[27].

The present study showed no significant relation between CEA and hepatomas, as the level of the former was normal in the sera of patients except for only 4% of them. Refaat[28] reported that CEA is important as a laboratory investigation in hepatoma patients to detect colon primaries if the tumour is suspected to be a secondary. Maussier et al[29] reported that AFP remains the best marker for HCC, and the only one effective in discrimination of HCC from cirrhosis. They also reported that CEA and CA 19-9 are of no use.

It is worth mentioning that carcinogenesis involves complex changes in the pattern of enzyme activities. The extent of enzyme alterations depends on the degree of differentiation. Hepatoma cells differ in some properties concerning carbohydrate metabolism. The kinetic properties of hexokinase are changed and some feedback control mechanisms that regulate glycolysis in normal tissue do not work in hepatoma[30].

The present study revealed that glycogen was depleted in liver tissue of hepatoma patients. Such finding agrees with those of Doris et al[31] who reported that properties such as loss of glycogen storing capacity might be essential elements for malignant progression. Chekulaev et al.[32] carried out a study on experimental liver cancer in rats and they reported that after implantation of tumour, the stores of

glycogen even exceeds those of control healthy rats, but in terminal stage of tumour growth depletion liver glycogen was observed. Also Morgan and Roth[33] reported that decrease of glycogen synthesis in human hepatoma is due to decrease ability of insulin stimulation.

In conclusion, we would say that environmental factors in Egypt play a great role in the aetiology of hepatomas. As it is clear from the present study that farmers who are exposed to chemicals, during their work such as insecticides, herbicides and fertilizers are highly suspected to have hepatomas. These farmers are also subjected to bilharziasis, antibilharzial drugs and hepatitis B viral infection, which was found to be highly related to hepatoma development. The most common hepatoma type among farmers was found to be HCC. Regarding the laboratory diagnosis for hepatoma cases, AFP is still considered the gold standard of tumour markers in hepatolgoy. 5'-Nucleotidase proved to be the best liver enzyme to test for hepatoma, and it may be considered as an important tool for diagnosing HCC.

Other liver enzymes were also elevated in hepatoma but inspite of their significant elevation, they could not be used as markers to differentiate malignant from bengin liver diseases. PAS stain was useful in supporting the diagnosis of hepatoma cases specially to differentiate the clear cell type.

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