A Cross Sectional Study of Hepatitis B, C, some Trace Elements, Heavy Metals, Aflatoxin B1 and Schistosomiasis in a Rural Population, Egypt

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ABSTRACT

Chronic liver diseases are disastrous to health. Many factors are associated with their prevalence, hence endemicity. These are mainly infectious, parasitic and toxic. A survey was conducted in a village south to Cairo. Large industries concerned with iron and steel industry, metals smelting, cement manufacturing and electric station were located north to the village. A systematic random sample of houses was selected. All individuals inside the houses were invited to share in the study. Sample size was 84 individuals. Hepatitis markers were done (HBsAg and anti-HCV antibodies). The levels of some heavy metals were assessed; which were lead, mercury, arsenic, aluminum, manganese, nickel, chromium and cadmium. Levels of some trace elements were assessed. These were copper, iron, selenium and zinc. Aflatoxin B1 was assessed in serum. Assessment of schistosomal circulating antigen and antibodies was carried out. Abdominal ultrasonograghy was done to assess liver condition. Univariate logistic regression analysis was done to assess the association between studied

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variables and HBsAg or anti-HCV sero-positive subjects. The association between studied variables and bilharzial or fatty liver, diagnosed by ultrasonography, were also assessed. The univariate logistic regression analysis revealed odds ratios at the following results. For HBsAg sero-positive subjects, aflatoxin B1, lead, chromium and schistosomal antigen and antibodies were higher than negative ones where odds ratios were; 6.2, 1.6, 1.6, 1.6 and 1.7, respectively. None of the variables showed statistically significant difference. For anti-HCV antibodies sero-positive subjects, aflatoxin B1 and chromium had the highest odds ratios among the studied variables, (odds ratios were 2.5 and 2.4, respectively). Bilharzial liver showed higher significant positivity of anti-HCV antibodies and insignificant decreased level of zinc than negative ones (odds ratios were 7.2 and 4.5, respectively). Fatty liver cases showed higher statistically significant positivity of anti-HCV antibodies and chromium than negative ones. Odds ratios were 8.0 and 7.1, respectively. Statistically significant lower level of aflatoxin B1 was shown in fatty liver than normal liver subjects. Multivariate logistic regression analysis for fatty liver showed that only anti-HCV antibodies sero-positivity had statistically significant odds ratio in comparison to chromium level and aflatoxin B1. It is concluded that some heavy metals, and Aflatoxin B1 had a definite association with liver diseases in the area under study. Having anti-HCV antibodies had a relation with fatty liver and with bilharzial liver more than having HBsAg. It is recommended that environmental management to factories nearby the village is urgently needed to decrease exposure to heavy metals. Prevention of hepatitis infection and aflatoxin exposure through different means is also recommended, other wise health care authorities would be confronted with unusual cases of HCC in the nearby future.

Keywords: hepatitis B, hepatitis C, trace elements, heavy metals, afltoxin, s.antigen, s.antibody, population survey.

INTRODUCTION

Chronic liver diseases (CLD) are considered an important health problem in Egypt.(1) They have a disastrous effect on health, hence economic potential of affected persons. Hepatitis B virus (HBV) infection showed a high prevalence in some communities in Egypt reaching up to 56.7%. However, infection was found in 6.7% who had HBsAg.(2) Unfortunately, hepatitis C virus (HCV) infection showed also

high prevalence. It was reported that the overall prevalence of anti-HCV antibody was 23.7%. (3) Moreover, a high percentage of post HBV (70% to 90% of cirrhotic patients) and post HCV (44%) associated cirrhosis progress to hepatocellular carcinoma (HCC) (4,5) Because of the fact that HCC is disastrous to health, epidemiological studies were much more concerned with it than with liver diseases in general. However, study of multiple factors associated with liver diseases in a community may unveil the prospect of future risk of HCC in this community.

Many risk factors are associated with liver disorders; these are infectious, parasitic, and environmental toxins. Synergism of more than one factor may enhance the process of liver damage. Several studies documented the associations and interactions between hepatitis, aflatoxins, especially aflatoxin B1, trace elements and heavy metals in the development of liver diseases. Risk factors of HCC in Egypt were studied.(6) It was proved that age over 60, farming, cigarette smoking, and occupational exposure to chemicals as pesticides were among risk factors for HCC. However, HBV and schistosomiasis were the major risk factors. Aflatoxin (one of the mycotoxins) had been found to play a role either alone or in combination with viral hepatitis in the development of HCC.(7,8) It was experimentally proved also that, hepatic failure may result from combined effect of schistosomiasis and aflatoxin B1 rather than single affection.(9)

Aflatoxin is well recognized as hepatotoxic. The relation between aflatoxin B1, genetic and environmental factors was studied. (10) The authors concluded that, there were relative contributions of environmental exposure and host susceptibility factors in the formation of aflatoxin B1 albumin adduct increasing the risk of liver cancer. Interaction between aflatoxin B1, plasma selenium, hepatitis B, genotypes of glutathione-S-transferase and antioxidants in the

development of HCC was studied.[11,12] These studies provided insight into the dietary and genetic factors influencing aflatoxin B1 in liver cancer development.

Trace elements are involved in chronic liver diseases because these elements may have a direct hepatic toxicity or may be decreased as a consequence of impaired liver function.[13] Selenium is a key nutrient in counteracting certain viral infections. [14] Selenium has several public health implications particularly in relation to chronic disease prevalence. It is important for healthy human response.[15] It was reported that plasma selenium concentration was reduced in patients with cirrhosis as compared to control.[16] Selenium and other antioxidants may play a role in slowing the progression of chronic hepatitis C infection to cirrhosis.[17] Decreased plasma selenium levels in patients with chronic hepatitis virus infection increased the risk of hepatocellular carcinoma.[18]

There is a key link between iron metabolism and pathophysiology of viral hepatitis. The presence of iron overload facilitated infection.[19] Increase in iron stores (increase in serum ferritin and transferrin iron saturation) leads to increased response to HCV infection [20], and progression of chronic hepatitis C.[21] Cirrhotic patients had a significant decrease of ferrous, zinc and selenium more than chronic hepatitis group.[13] It was found that liver impairment in HCV related chronic liver disease is the main cause of blood decrease in selenium and zinc, independently of the nutritional status, whereas the associated malnutrition affects the ferrous levels only.[22]

Zinc levels of the patients with chronic hepatitis, cirrhosis and HCC were lower than those of controls, while copper levels were higher in patients with cirrhosis and HCC than that of chronic hepatitis patients and controls.[23, 24] There was also an increase in serum copper level in

patients with acute hepatitis B.[25] Low liver zinc and high liver copper determine amount of fibrosis.[26] Copper accumulation in fibrotic liver caused by chronic hepatitis C may contribute to hepatic injury and copper content has been increased with the progression of hepatic fibrosis.[27] Zinc decreases the toxic effect of copper on liver.[28,29]

Intestinal absorption of both iron and manganese are increased in iron deficiency. Iron deficiency may therefore increase the susceptibility to manganese neurotoxicity. The log of erythrocyte manganese concentration was inversely correlated with all measures of iron status. The findings confirm the association of manganese with the development of the pallidal hyperintensity in patients with liver disease. Increased excretion of zinc and iron in cirrhosis leads to decreased excretion of manganese resulting in increase in manganese. The authors recommended that patients with chronic liver disease should avoid manganese supplements without concurrent iron supplementation.[30]

Hepatotoxic effect of organophosphorous insecticides diminished serum and hepatic zinc levels, serum iron, but increase serum copper levels.[31]

Nearly most of heavy metals had toxic effect on liver. Lead level in blood increased with alcoholic liver disease with or without cirrhosis.[32] An increase in lead level in liver disease was reported by another study.[33]

Mercury had a toxic effect on liver.[34] Arsenic induced liver hyperplasia.[35] Cadmium toxicity to liver was studied.[36] It acts in a dose dependent manner.[37] Chromium had hepatotoxic effect.[38] A definite correlation was found between cadmium, chromium and nickel and chronic liver diseases.[39] Nickel protects against cadmium

hepatotoxicity.[40] Selenium exerted a protective effect on liver against the toxicity of cadmium and mercury.[41] Zinc also had a protective action against mercury toxicity.[42]

Immunodiagnostic investigation for *S.mansoni* infection such as circulating antigen and antibody with other clinical data such as abdominal ultrasonography allow calculating an accurate estimate of *S.mansoni* prevalence.[43]

AIM OF THE STUDY

The aim of this work is to assess some factors that may be associated with some common liver diseases in a community based study. Common liver diseases were hepatitis B, C, bilharzial or fatty liver. These factors are some heavy metals, some trace elements, aflatoxin B1 and *Schistosoma mansoni* infection.

MATERIAL AND METHODS

Study area:

El Shobak El Sharki village related to El Saf district, Guiza governorate was the site of this study. The village lies in the south of Guiza governorate at the east side of the Nile as a natural connection to Cairo. It lies nearby many factories, iron and steel, metal melting including lead, electricity station, cement industry and petroleum pipes company.

Study population and sample selection:

This piece of work was a part of a large survey of the village done to screen liver, renal and urinary tract diseases. Population was mainly involved in agriculture. Total population size was about 27,600 persons. Some population works in nearby factories. Systematic random sample of houses (about 90 houses) was selected for the original survey. All households were invited to share in the study and informed about the procedures that would be done for them. Consent was obtained. Because of financial constraints, only 84 subjects (representing 15 houses from the total surveyed ones), who fulfilled the required parameters for this work were studied. The 15 houses were selected randomly representing five geographical locations of the village (north, south, east, west and center).

Laboratory investigations:

The following assessments were done by the following techniques:

1. Serum aflatoxin B1 by Ridascreen Aflatoxin B1 enzyme immunoassay for the quantitative analysis of aflatoxin B1 (Reichert *et al.,* (44)).

2. Heavy metals and trace elements were assessed using flameless atomic absorption model 460, Perkin Elmer Company as follows:
   - Serum aluminum (Al), copper (Cu), selenium (Se), iron (Fe) and zinc (Zn).
   - Whole heparinized blood lead (Pb), manganese (Mn), nickel (Ni) and chromium (Cr).
   - Urine cadmium (Cd), arsenic (As) and mercury (Hg).
3. Hepatitis markers:
   The following markers were used:
   1- Hepatitis B surface antigen (HBsAg) by ELISA assay using Merux HBsAg version 3 kit.

2- Hepatitis C antibodies (Anti-HCV Ab) by ELISA assay using Merux Anti-HCV version 4 kit.

3- Schistosomal immunodiagnostic tests:

Circulating schistosomal antigen (Schist. Ag) detection using MAb Sandwitch ELISA system and measurement of anti-schistosomal antibody (Schist. Ab) by indirect ELISA were carried out (Engvall and Perlmann, [45]). From a panel of 18 monoclonal antibodies prepared against *S.mansoni* soluble egg antigen at the Immunology Department, Theodor Bilharz Research Institute, MAb 7 F1/6B was selected. It is an IgM which recognizes an antigen with repeating epitope on different stages of life cycle of *S.mansoni* and *S.haematobium* (Nourel Din *et al.,* [46]). It was employed as both antigen capturing and for detecting antibody. The lower detecting limit of this MAb is <1ng/ml of *S.mansoni* soluble antigen (Mahmoud *et al.*, [47]).

Clinical assessment:

Abdominal ultrasound examination was performed using portable machine (Hitachi EUB-200). All subjects were asked for overnight fasting. Full assessment of liver, gall bladder, spleen, kidneys, urinary bladder and prostate was done. For this study, only liver findings were processed.

Statistical analysis:

Data were processed using SPSS version 11 software program. Means and standard deviations were computed for age. Logistic regression analysis was done. Univariate logistic regression analysis was made between measured variables supposed to have an association with HBsAg, anti-HCV antibodies and liver affection detected by ultrasonograghy, separately. These variables were aflatoxin B1, heavy metals (lead, mercury, arsenic, cadmium, nickel, chromium, manganese

and aluminum), trace elements (copper, zinc, selenium and iron), schistosomal antigen and schistosomal antibody. Variables having numerical values were expressed as median and range. All variables were classified to binomial variables according to the cutoff point. Zinc and selenium having values less than reference ranges were considered as abnormal. Those having values equal or more than reference ranges were considered as normal. Initially, copper and iron were classified into those having values below reference range, those having values within reference range and those having values above reference range. As, no single case was detected having levels below reference range for these two trace elements, subjects were classified as those within the reference range (normal) and those having levels above the reference range (abnormal). For heavy metals, those having values more than the reference ranges were considered as abnormal. Those having values equal or less than the reference ones were considered normal. Chronic liver disease, classified as fatty or bilharzial liver, was diagnosed based on abdominal ultrasonography. Odds ratios (ORs), 95% confidence interval (CI) of odds ratios more than the unity and P values were calculated by univariate logistic regression analysis. Odds ratios with significant P values (≤ 0.05) were considered statistically significant. Only multiple significant odds ratios were found with fatty liver. So, multivariable logistic regression analysis was computed for factors associated with fatty liver namely chromium exposure, aflatoxin B1 and anti-HCV antibodies positivity. Odds ratio more than the unity means that odds of having affected level of studied variables in a positive parameter for hepatitis or liver affection detected by ultrasonograghy are more than odds of having affected level in negative hepatitis or liver are more than odds of having affected level in negative hepatitis or liver affection subjects.

Age was used as such to calculate a series of correlations between age and exposure. Also, age was classified into those ≤ 15 years and older age subjects. Non parametric testing using Chi-square test was also used to show the relation of age with different studied parameters. The 15 years old was considered a suitable age to become active outside the house and may be indulged in a professional work.

RESULTS

Household members examined were 84 persons having all age groups and both sexes. Mean± SD age was 26.3± 17.4, median was 20 years of age, range varied from 5 to 73. Male to female ratio was 0.65. Males represented 39.5% and females 60.5% of the total examined. Unexpectedly, HBsAg sero-positivity alone was 30.0% and anti-HCV antibodies sero-positivity was 14.3%. Combined HBsAg and anti-HCV antibodies sero-positivity was 7.0%. Percent negative for both HBsAg and anti-HCV antibodies was 48.7%. Abdominal ultrasonograghy revealed that of examined, 57.3% were normal, 12.0% had bilharzial liver, and 14.7% had fatty liver. Only one patient had cirrhosis. The rest of subjects had non related conditions. Percentage of HBsAg in either of bilharzial, fatty or cirrhotic liver patients was 33.3%, while that of anti-HCV-antibodies was 42.1%. According to age groups, anti-HCV sero-positive antibodies were found only in age group more than 15 years of age (P = 0.003; the difference was statistically significant). In contrary HBsAg sero-positivity was more in age group ≤15 (57.1%); but it was 27.5 % in age group >15 (OR= 3.5, 95% CI= 1.2-10.2, P value was 0.01; the difference was statistically significant). In age group ≤15, bilharzial liver was present in 15.0% and in 18.8% of age group >15 (the difference was statistically insignificant). Fatty liver showed 5.6% positivity rate in age group ≤15 and 27.8% in age group >15 (OR = 6.5, 95% CI= 0.8-55.8, P = 0.056; the difference was statistically insignificant).

Zinc level was negatively correlated with age (r = -0.2, P = 0.08), all those having decreased zinc level were at age group >15 (P = 0.03). Aluminum was positively correlated with age (r = 0.3, P = 0.04). Other studied trace elements and heavy metals had very weak correlations.

Anti-Schistosomal antibodies were found in 60.9% of subjects of age group ≤15 and in 51.3% of age group >15; the difference was statistically insignificant. But circulating *Schistosoma* antigen was found in 87.5% of subjects at age group ≤15 and in 67.4% of those at age group >15 (the difference was also statistically insignificant; P = 0.07).

As regards effect of sex on studied variables, the only significant heavy metal that was found in higher percentage of females than males was chromium (58.5% of females had elevated level vs. 29.2% of males, with OR = 3.4, CI =1.2-10.1, P = 0.02). Anti-HCV antibodies were present in a comparable percentage in males and females (20.7% of males vs. 19.6% of females. On the other hand HBsAg was present in 50.0% of males and 28.3% of females with OR = 2.5. A percentage of 27.3% of males and 10.0% of females had bilharzial liver, with OR = 3.4. Of females, 25.0% had fatty liver and that of males were 11.1%, OR = 2.7. All the differences were statistically insignificant.

Table 1 shows median and range of all studied numerical variables. Selenium was higher than the reference value. Mercury in all subjects was within the reference range. Cadmium and lead had medians that were higher than the reference range with different degrees of elevation, while, their ranges showed that some cases were within reference range. All other variables showed that some cases had abnormal levels that differed according to hepatitis condition. The table shows also that aflatoxin B1 median level was elevated with very high upper limit of the range in all groups.

Table (1): Median and Range of Trace Elements, Heavy Metals and Aflatoxin B1 in Patients with Hepatitis Serologic Findings.

Variable	Negative HBsAg and Anti-HCV Ab	HBsAg positive	Anti-HCV Ab positive	Combined infection	Reference value
Trace elements:					
Copper	93.0 (79.0-131.0)	96.0 (88.0-127.0)	99.0 (83.0-120.0)	93.0 (91.0-95.0)	80-110/µg dL
Zinc	91.0 (65.0-125.0)	92.0 (76.0-110.0)	92.5 (69.0-109.0)	89.5 (88.0-91.0)	80-120/µg dL
Selenium	16.6 (12.8-22.2)	14.9 (13.3-18.4)	15.9 (13.6-17.8)	17.3 (16.1-18.4)	5.7-7.4/µg dL
Iron	111.5 (99.0-133.0)	108.0 (97.0-122.0)	108.5 (100.0-117.0)	108.5 (108.0-109.0)	65-120/µg dL
Heavy metals:					
Aluminum	47.0 (29.0-66.0)	44.0 (27.0-60.0)	40.0 (29.0-71.0)	32.5 (29.0-36.0)	14-62/µg dL
Cadmium	2.5 (1.7-3.3)	2.4 (1.7-3.2)	2.5 (1.9-3.9)	2.5 (2.3-2.7)	0.9-2/µg L
Arsenic	50.0 (32.0-69.0)	51.0 (26.0-84.0)	44.0 (41.0-69.0)	48.5 (42.0-54.0)	≈80/µg L
Mercury	99.0 (39.0-168.0)	111.0 (45.0-151.0)	127.0 (91.0-169.0)	120.0 (95.0-142.0)	100-300/µg L
Chromium	28.0 (22.0-44.0)	30.0 (20.0-39.0)	32.0 (23.0-39.0)	31.0 (23.0-38.0)	20-30/µg L
Lead	38.5 (27.0-53.0)	39.0 (27.0-47.0)	38.5 (25.0-54.0)	43.5 (41.0-52.0)	10-38/µg dL
Manganese	7.9 (5.7-13.2)	7.8 (5.7-11.2)	7.6 (5.8-11.3)	8.3 (6.5-12.8)	7-12/µg L
Nickel	4.6 (2.9-6.6)	4.1 (2.9-7.1)	3.7 (2.9-5.1)	5.0 (3.8-6.5)	4.8/µg L
Aflatoxin B1	45.0 (0.0-275.0)	65.0 (0.0-220.0)	37.5 (0.0-270.0)	52.5 (25.0-187.5)	13.6-36.2 ng/kg

Table 2 shows the same pattern as table (1) except that the median of lead was within the reference range and median of chromium was higher than the reference range in fatty liver cases.

In anti-HCV antibodies sero-positive subjects, there was very weak negative correlation between iron and manganese (r = -0.04, P = 0.9). There was also negative correlation between copper and selenium levels (r = -0.54, P = 0.07) and positive correlation between zinc and iron levels (r = 0.36, P = 0.26). There was also positive correlation between zinc and copper (r = 0.37, P = 0.24). As regards heavy metals, there was statistically significant positive correlation between lead and manganese levels (r = 0.6, P = 0.04) and selenium and nickel levels (r = 0.75, P = 0.01). In HBsAg sero-positive patients also, copper showed negative correlation with selenium (r = -0.39, P = 0.12) and positive correlation with zinc (r = 0.38, P = 0.14). In subjects with bilharzial liver, no distinguished correlations were found. Fatty liver subjects showed negative correlation between copper and selenium levels (r = -0.32, P = 0.40) and positive correlation between copper and zinc (r = 0.66, P = 0.05; statistically significant). Also, iron showed positive correlation with selenium (r = 0.62, P = 0.19).

Table 3 shows that elevated iron or copper or decreased zinc levels showed odds ratios lower than the unity in HBsAg sero-positive subjects. Cadmium, lead, chromium and nickel had high percentages in all subjects either positive or negative in the same mentioned order. But those having elevated lead and chromium had higher percentages within HBsAg positive cases than negative ones. Odds ratios were 1.6 (95% CI= 0.5-5.5) and 1.6 (95% CI= 0.5-5.3), respectively, but both differences were statistically insignificant.

Table (2): Median and Range of Trace Elements, Heavy Metals and Aflatoxin B1 in Subjects with Chronic Liver Disease (Bilharzial or Fatty Liver) as Diagnosed by Abdominal Ultrasonographgy.

Variable	Normal liver	Bilharzial liver	Fatty liver	Reference value
Trace elements:				
Copper	97.0 (79.0-131.0)	87.0 (83.0-124.0)	95.0 (83.0-127.0)	80-110/µg dL
Zinc	91.0 (76.0-125.0)	98.0 (74.0-101.0)	91.0 (69.0-109.0)	80-120/µg dL
Selenium	16.6 (12.8-22.2)	17.7 (15.2-21.1)	16.1 (14.8-18.3)	5.7-7.4/µg dL
Iron	111.0 (97.0-133.0)	111.5 (109.0-114.0)	107.0 (101.0-122.0)	65-120/µg dL
Heavy metals:				
Aluminum	43.0 (27.0-71.0)	53.0 (41.0-56.0)	42.0 (29.0-54.0)	14-62/µg dL
Cadmium	2.5 (1.7-3.9)	2.3 (1.9-2.6)	2.5 (1.9-3.3)	0.9-2/µg L
Arsenic	50.0 (26.0-84.0)	44.0 (29.0-66.0)	49.5 (42.0-64.0)	≈80/µg L
Mercury	113.0 (45.0-168.0)	101.0 (75.0-151.0)	85.0 (38.0-148.0)	100-300/µg L
Chromium	28.0 (20.0-44.0)	31.0 (23.0-36.0)	33.0 (24.0-41.0)	20-30/µg L
Lead	39.0 (27.0-54.0)	40.0 (29.0-53.0)	36.0 (25.0-52.0)	10-38/µg dL
Manganese	7.9 (5.7-13.2)	7.8 (5.9-8.8)	7.5 (6.8-12.8)	7-12/µg L
Nickel	4.4 (2.9-7.1)	4.7 (3.3-5.3)	3.6 (2.9-5.9)	4.8/µg L
Aflatoxin B1	50.0 (0.0-325.0)	25.0 (0.0-200.0)	22.5 (0.0-160.0)	13.6-36.2 ng/kg

Those having elevated aflatoxin B1 had higher percentage (95.2%) within HBsAg positive cases than negative ones (76.5%). Odds ratio was 6.2 (95% CI =0.7-53.3), but the difference was statistically insignificant.

Percentages of those having *Schistosoma* antigen were higher in HBsAg positive cases than negative ones. Percentages were 83.3% and 75.9%, respectively; OR= 1.6 (95% CI= 0.4-7.2). Also, those having *Schistosoma* antibodies were more higher within HBsAg positive subjects than negative ones (64.7% vs. 52.0%; OR = 1.7, 95% CI= 0.5-6.0), the differences were statistically insignificant. A percentage of 81.0% of those positive for HBsAg had either *Schistosoma* antigen or antibodies.

For anti-HCV Ab positive subjects, iron level was not elevated in any case. Copper was elevated in 30.0% of those having anti-HCV Ab positive sera, but it was elevated also in 32.0% of those having negative sera for both HBsAg and anti-HCV Ab. Decreased zinc level was not associated with anti-HCV Ab sero-positivity. Cadmium, lead, chromium and nickel had high percentages in both anti-HCV Ab positive or negative subjects. Chromium and aluminum were the only two heavy metals that show association with anti-HCV Ab sero-positivity. For chromium, odds ratio was 2.4, 95% CI= 0.5-12.3. For aluminum, odds ratio was 1.3; 95%CI= 0.1-16.0. The differences were statistically insignificant.

Aflatoxin B1 was more elevated in anti-HCV Ab positive cases than negative ones (88.9% vs. 76.5%, respectively, OR = 2.5, 95% CI= 0.3-22.8, the difference was statistically insignificant).

Presence of *Schistosoma* antigen was not associated with anti-HCV Ab sero-positivity, while, *Schistosoma* antibodies were slightly higher in positive than negative ones. (Odds ratio was 1.2, 95% CI= 0.2-6.7). A percentage of 60.0% of anti-HCV Ab positive sera had either positive *Schistosoma* antigen or antibody.

Combined sero-positivity of HBsAg and anti-HCV Ab showed associations with elevated manganese, chromium and nickel levels. Odds ratios and 95% CI were 2.8 (0.2-42.0), 1.5 (0.2-12.0) and 1.4 (0.2-12.0), respectively. The differences were statistically insignificant. Aflatoxin B1, lead and cadmium showed 100.0% positivity in combined HBsAg and anti-HCV Ab positive subjects.

The association between normal, bilharzial or fatty liver and studied variables is shown in Table 4. Bilharzial and fatty liver were diagnosed on the basis of abdominal ultrasonography. The association

Table (3): Percentage Distribution, Odds Ratios and 95% Confidence Intervals of Studied Trace Elements, Heavy Metals, Aflatoxin B1, *Schistosoma* Antibodies, and Antigen among Patients with Hepatitis Serologic Findings.

Variable	Negative	HBsAg positive	Anti-HCV Ab positive	Combined infection
Trace elements:				
Copper (%)	32.0	13.3	30.0	0.0
OR (95% CI)	-	0.3 (0.06-1.8)	0.9 (0.2-4.5)	-
Zinc (%)	16.0	13.3	10.0	0.0
OR (95% CI)	-	0.8 (0.1-5.1)	0.6 (0.06-6.0)	-
Iron (%)	11.1	7.7	0.0	0.0
OR (95% CI)	-	0.7 (0.05-8.2)	-	-
Heavy metals:				
Aluminum (%)	8.0	0.0	10.0	0.0
OR (95% CI)	-	-	1.3 (0.1-16.0)	-
Cadmium (%)	88.5	76.5	88.9	100.0
OR (95% CI)	-	0.4 (0.08-2.2)	1.04 (0.1-11.5)	-
Arsenic (%)	0.0	5.9	0.0	0.0
OR (95% CI)	-	-	-	-
Chromium (%)	40.7	52.6	62.5	50.0
OR (95% CI)	-	1.6 (0.5-5.3)	2.4 (0.5-12.3)	1.5 (0.2-12.0)
Lead (%)	57.1	68.4	50.0	100.0
OR (95% CI)	-	1.6 (0.5-5.5)	0.7 (0.2-3.6)	-
Manganese (%)	10.5	0.0	0.0	25.0
OR (95% CI)	-	-	-	2.8 (0.2-42.0)
Nickel (%)	42.1	18.8	12.5	50.0
OR (95% CI)	-	0.3 (0.07-1.5)	0.2 (0.02-1.9)	1.4 (0.2-12.0)
Aflatoxin B1 (%)	76.5	95.2	88.9	100.0
OR (95% CI)	-	6.2 (0.7-53.3)	2.5 (0.3-22.8)	-
Schist. Ab (%)	52.0	64.7	57.1	50.0
OR (95% CI)	-	1.7 (0.5-6.0)	1.2 (0.2-6.7)	0.9 (0.1-7.6)
Schist. Ag (%)	75.9	83.3	57.1	25.0
OR (95% CI)	-	1.6 (0.4-7.2)	0.4 (0.08-2.4)	0.1 (0.01-1.2)

N.B. All studied variables are divided into those within reference range and above this range except zinc.

Percentages refer to subjects having abnormal levels of studied variables within normal, HBsAg, anti-HCV antibodies or combined sero-positive subjects.

between studied variables and normal liver in comparison to bilharzial liver shows that the percentage of those having elevated copper level within bilharzial liver subjects was 40.0% vs. 19.4% within subjects having normal liver (OR= 2.8, 95% CI= 0.4-20.5, the difference was statistically insignificant). Iron level was elevated in 7.4% of normal liver subjects and in none of bilharzial liver subjects, but the difference was statistically insignificant. Zinc level was low in 40.0% of bilharzial liver subjects and in only 12.9% of normal liver subjects, (OR = 4.5, 95% CI = 0.6-35.8, the difference was statistically insignificant).

Some heavy metals showed increased percentages in both normal and bilharzial liver subjects; those were chromium, cadmium, nickel and lead. Their percentages were more among bilharzial than normal liver subjects. Their odds ratios and 95% CI were 1.8 (0.4-8.3) for chromium, 1.6 (0.2-15.7) for cadmium, 1.6 (0.2-11.0) for nickel and 1.4 (0.3-8.2) for lead. All the differences were statistically insignificant.

Aflatoxin B1 was elevated in 90.7% of normal liver subjects and in 77.8% of bilharzial liver subjects; the difference was statistically insignificant.

Subjects having *Schistosoma* antibodies constituted 54.5% of normal liver subjects and 42.9% of bilharzial liver subjects, the difference was statistically insignificant. Those having *Schistosoma* antigen constituted 83.3% of normal liver subjects and 75.0% of bilharzial liver subjects, the difference was statistically insignificant. All those having bilharzial liver had either positive *Schistosoma* antigen or antibody.

Subjects having HBsAg constituted 44.4% of bilharzial liver subjects in comparison to 39.5% of normal liver subjects (OR = 1.2, 95% CI = 0.3-5.3, the difference was statistically insignificant). The striking finding was that percentage of anti-HCV Ab positivity within bilharzial liver

subjects was 37.5% in comparison to 7.7% of normal liver subjects (OR = 7.2, 95% CI = 1.1-46.0; the difference was statistically significant).

Comparing fatty to normal liver in relation to other studied factors showed that those having elevated copper level within fatty liver subjects represented 33.3% compared to 19.4% of normal liver subjects (OR = 2.1, 95% CI= 0.4-10.8, the difference was statistically insignificant). Also, 16.7% of fatty liver subjects had elevated iron level in comparison to 7.4% of normal liver subjects (OR = 2.5, 95% CI = 0.2-33.2, the difference was statistically insignificant). Percentages of those having decreased zinc level were comparable in normal and fatty liver subjects.

Those having elevated chromium, cadmium and manganese had higher percentages in fatty liver subjects than normal liver subjects. Still cadmium and chromium had high percentages in either fatty or normal liver subjects. For chromium, OR = 7.1, 95% C = 1.3-38.4, the difference was statistically significant. Cadmium as mentioned before had elevated median values in all groups, yet it was more in fatty liver subjects than normal ones, OR = 2.1, 95% CI = 0.2-19.7, the difference was statistically insignificant. Elevated manganese level within fatty liver subjects had an odds ratio of 2.8, 95% CI= 0.2-37.0, the difference was statistically insignificant. Lead level showed a pattern that was different from above results. Percentage of elevated lead level was higher in normal liver subjects than those having fatty liver (67.6% vs. 40.0%, respectively; the difference was statistically insignificant).

Aflatoxin B1 was elevated in 90.7% normal liver subjects and in 63.6% of fatty liver ones (OR = 0.2, 95% CI = 0.04-0.9; the difference was statistically significant).

Those having *Schistosoma* antibodies constituted 54.5% of normal liver subjects and 88.9% of fatty liver ones, (OR = 6.7, 95% CI = 0.7-59.5,

the difference was statistically insignificant). Those having *Schistosoma* antigen constituted 83.3% of normal liver subjects and 60.0% of fatty liver ones, the difference was statistically insignificant. All those having fatty liver had either positive *Schistosoma* antigen or antibody.

Those having HBsAg constituted 18.2% of fatty liver subjects in comparison to 39.5% of normal liver subjects (the difference was statistically insignificant). The percentage of anti-HCV Ab positivity within fatty liver subjects was 40.0% in comparison to 7.7% of normal liver ones (OR = 8.0, 95% CI = 1.4-45.1, the difference was statistically significant).

Table 5 shows the multivariate logistic regression analysis for fatty liver cases. Variables in the analysis were anti-HCV antibodies, aflatoxin B1 and chromium level. Anti-HCV antibodies in fatty liver subjects was the only variable that showed statistically significant odds ratio (OR =9.0, 95% CI = 1.3 - 63.7).

DISCUSSION

Some factors that may be associated with some liver diseases in an Egyptian rural community were studied. These were some heavy metals, some trace elements, aflatoxin B1 and *S. mansoni* infection. Diagnosis depends on non-invasive techniques as detection in body biological fluids for heavy metals and trace elements according to type detected, abdominal ultrasonography, circulating schistosomal antigen and antibodies, hepatitis markers; HBsAg and anti-HCV antibodies. Abdominal ultrasonography was used to diagnose liver condition. The heavy metals and trace elements which are supposed to have hepatic

Table (4): Percentage Distribution, Odds Ratios and 95% Confidence Intervals of Studied Trace Elements, Heavy Metals, Aflatoxin B1, *Schistosoma*

Antibodies, Antigen, Hbsag and Anti-HCV Antibodies among Subjects with Normal, Bilharzial or Fatty Liver.

Variable	Negative	Bilharzial liver	Fatty liver
Trace elements:			
Copper (%)	19.4	40.0	33.3
OR (95% CI)	-	2.8 (0.4-20.5)	2.1 (0.4-10.8)
Zinc (%)	12.9	40.0	11.1
OR (95% CI)	-	4.5 (0.6-35.8)	0.8 (0.1-8.7)
Iron (%)	7.4	0.0	16.7
OR (95% CI)	-	-	2.5 (0.2-33.2)
Heavy metals:			
Aluminum (%)	3.2	0.0	0.0
OR (95% CI)	-	-	-
Cadmium (%)	81.3	87.5	90.0
OR (95% CI)	-	1.6 (0.2-15.7)	2.1 (0.2-19.7)
Arsenic (%)	3.1	0.0	0.0
OR (95% CI)	-	-	-
Chromium (%)	36.1	50.0	80.0
OR (95% CI)	-	1.8 (0.4-8.3)	7.1 (1.3-38.4)*
Lead (%)	67.6	75.0	40.0
OR (95% CI)	-	1.4 (0.3-8.2)	0.3 (0.1-1.4)
Manganese (%)	6.7	0.0	16.7
OR (95% CI)	-	-	2.8 (0.2-37.0)
Nickel (%)	30.0	40.0	16.7
OR (95% CI)	-	1.6 (0.2-11.0)	0.5 (0.04-4.6)
Aflatoxin B1 (%)	90.7	77.8	63.6
OR (95% CI)	-	0.4 (0.1-2.3)	0.2 (0.04- 0.9)*
Schist. Ab (%)	54.5	42.9	88.9
OR (95% CI)	-	0.6 (0.1-3.2)	6.7 (0.7-59.5)
Schist. Ag (%)	83.3	75.0	60.0
OR (95% CI)	-	0.6 (0.1-3.7)	0.3 (0.1-1.4)
HBsAg (%)	39.5	44.4	18.2
OR (95% CI)	-	1.2 (0.3-5.3)	0.3 (0.1-1.8)
Anti-HCV Ab (%)	7.7	37.5	40.0
OR (95% CI)	-	7.2 (1.1-46.0)*	8.0 (1.4-45.1)*

N.B. All studied variables are divided into those within reference range and above this range except zinc.

Percentages refer to subjects having abnormal levels of studied variables among normal, fatty or bilharzial liver subjects.

** Statistically significant difference was found.*

Table (5): Output of Multivariate Logistic Regression Analysis for Fatty Liver Subjects.

Variable	OR	95 % CI	Significance
Chromium	1.1	0.9-1.3	0.31
Aflatoxin B1	0.1	0.01-1.1	0.06
Anti-HCV antibodies	9.0	1.3-63.7	0.03*

** Statistically significant difference was found.*

effect were assessed in a randomly selected sample of population from El Shobak El Sharki village, Guiza governorate. These were lead, aluminum, mercury, chromium, cadmium, arsenic, nickel, and manganese. Trace elements studied were copper, zinc, selenium, and iron.

Although selenium exceeded the reference range, it is a protective nutrient where its deficiency is harmful. All heavy metals exposures were supposed to be due to industrial exposure from the nearby large industries. Chromium was associated with stainless steel industry. Lead exposure from lead smelting and cadmium as a byproduct from lead smelters. Nickel resulted mainly from petroleum industry. (48) Aflatoxin B1 exposure usually resulted from dietary sources. (49) In this study increase age was only associated with decreased zinc level. Decreased zinc level was also associated with bilharzial liver which had no relation with age. So, decreased zinc level with age could not be explained by the presence of any of the studied variables. Aluminum also showed positive correlation with age. But aluminum was within the reference range in all subjects and showed no relation with any of the studied variables.

The age distribution in relation to hepatic affection showed that anti-HCV antibodies were found only in age group >15 years old, while, HBsAg was found more in age group ≤ 15 than age group >15 years old (OR = 3.5, the difference is statistically significant). This may

threw question about the validity of HBV immunization program for infants or immunity against HBV infection in smaller age groups. Bilharzial liver affection was comparable in either age groups while fatty liver was more in older age group. The association between fatty liver and age may be explained by the association between fatty liver and anti-HCV antibodies which were found in older age group.

The assumption that those having hepatic conditions are more susceptible to exposure to hepatotoxic metals, aflatoxin B1, having more affected levels of some related trace elements, and more associated with schistosomiasis than normal subjects was investigated and shown in Tables 3 and 4. Anti-HCV antibodies were somewhat more frequent in fatty liver cases (40.0% with OR = 8.0) than bilharzial liver group (37.5% and OR=7.2, table 4). This result coincides with that of Raziu *et al*., (2004) as regards association of fatty liver and chronic hepatitis C. (50) The findings of the present study go in accordance with results of Strickland *et al*., (2002) who found in an Egyptian study that 60% of patients with chronic liver diseases were anti-HCV antibodies positive compared to 6% having HBsAg.(1) They concluded that the predominant potential cause of chronic liver disease was infection with HCV. In the present study those having bilharzial liver had more risk to have HBsAg than fatty liver (OR=1.2 vs. 0.3, respectively, table 4). Another finding was reported by Kamel *et al*.,(1994) where no association was found between ultrasonographically–determined schistosomal hepatic fibrosis and HBsAg or anti-HCV sero-positivity. (51)

Table 3 showed that schistosomal antibody was found to be associated with the presence of HBsAg more than anti-HCV antibodies. Schistosomal antibodies were found in 64.7% of HBsAg positive subjects, 57.1% of anti-HCV Ab positive ones, and 52.0% of negative ones (ORs = 1.7 & 1.2, respectively). They were also found in 50.0% of

those having combined infection. El Zayadi *et al*., (1997) reported that HCV antibodies were significantly higher in schistosomal antibody positive Egyptians.(52) Also, *Schistosoma* antibodies were found in 92.0% of the anti-HCV positive HBsAg negative sera of HCC patients by another study. (53) However, Kamel *et al*., (1994) found no epidemiological association between *S. mansoni* infection or the intensity of *S. mansoni* infection diagnosed parasitologically and the presence of HBsAg or HCV antibodies.(51) The controversy of the results may be due to dependence of parasitological versus immunolgical techniques in diagnosis of *Schistosoma* infection and use of different types of study design.

Fortunately in the present study, selenium was normal or even exceeding the reference value in all studied subjects. It is a key nutrient in counteracting certain viral infections.(14) It also decreases the prevalence of chronic disease. (15) However, the prevalences of HBsAg, anti-HCV antibodies or combined infection sero-positive subjects, fatty liver and bilharzial liver, as found in this study, were high (30.0%, 14.3, 7.0%, 14.7% & 12.0%, respectively). It may be speculated that, it had a minor role in counteracting viral hepatitis in this community or the spread of viral hepatitis was so high and the protection by selenium keep the prevalence at this level not higher. Most studies revealed selenium reduction with cirrhosis. (16) In the present study of cross sectional survey of households, there was only one case of cirrhosis which did not permit to study the relation between selenium level and cirrhosis.

Decreased zinc level was an associating factor in bilharzial liver not in hepatitis subjects in this study as odds ratio was 4.5 but not significant (Table 4). More decrease in zinc in cirrhotics than chronic hepatitis was reported. (13) In contrary to the present results, many

authors reported decrease in zinc level with chronic viral hepatitis.[19, 23,54] The difference in the results may be due to different degrees of liver affection between the present study and other studies, as only one case of cirrhosis was detected in the present study. Attributing factors to pathological stage of liver affection may be high selenium and normal zinc levels in most of studied population.

Iron was normal or above the reference range in all subjects, denoting that liver pathology was not so severe to lower iron level. [13] In the present study, increased iron was found in 16.7% of fatty liver subjects versus 7.4% of normal liver subjects (Table 4). Increased iron stores was claimed to be a risk factor for contracting HCV. [20] This situation needs further studies among the Egyptian population depending on using suitable parameters that could be measured denoting the condition of iron stores.

As manganese level is negatively associated with iron level as shown by a previous study [30], it was not suspected to increase in the present study as iron level was normal or high, not low. However, it constituted an associating factor for the presence of combined HBsAg and fatty liver (Tables 3 and 4). It was found that a very weak negative correlation was found between manganese and iron in anti-HCV antibodies sero-positive cases (r = - 0.04). Sharada *et al*., (2001) found also that there was no significant relation between serum manganese in apparently healthy females and liver function tests.[54]

Copper was normal in most of the studied subjects. It was slightly higher than normal in some subjects (Tables 3 and 4). Bilharzial and fatty liver subjects showed increased copper level more than normal subjects (Table 4). However, normal serum zinc and copper

concentration levels were found in patients with hepatopathies when compared to controls. (29) The relation of copper level and liver function in a study on apparently healthy Egyptian females was proved to be insignificantly related to liver enzymes and insignificantly related to total proteins. (54)

Trace elements to be affected with bilharzial and fatty liver were copper, zinc and/or iron which may be of diagnostic and prognostic importance. Also, the correlations between selenium (with its high level) and other trace elements may explain the somewhat normality of the present results.

Heavy metals assessment revealed that lead level was high (Tables 1 and 2). However, it constituted an associating factor for HBsAg, combined sero-positivity of HBsAg and anti-HCV antibodies or bilharzial liver cases only (Tables 3 and 4). These results were comparable to those of Bortoli *et al.*, (1983).(33)

Mercury level was normal in all subjects. However, it did not constitute an associating factor due to initial non exposure or protective effect of selenium. (41) This protective effect did not include cadmium due to initial high exposure (Tables 1 and 2). There was no association of age found in this study with all trace elements or heavy metals except for aluminum and zinc as shown in results.

Tables 3 and 4 showed that chromium had odds ratio more than 1 in anti-HCV antibodies, HBsAg sero-positive or combined infection subjects and in those having bilharzial liver. Those having fatty livers had a significant increase in chromium level with odds ratio 7.1. This denoted that risk of exposure to chromium was associated with all liver diseases but fatty liver cases were the more exposed. These results may be in part due to high initial level and in another part to liver affection

by hepatitis or schistosomiasis. It was claimed to be hepatotoxic. [38] It was followed by cadmium and lead that had associations in liver affection cases. Age had only related to hepatitic or liver affection not to heavy metal exposure.

Increase in nickel level had occurred in combined HBsAg and anti-HCV antibodies sero-positivity and bilharzial liver subjects only, not in other studied liver diseases (Tables 3 and 4). The cause may be related to the degree of pathology of the liver in those subjects.

Aflatoxin B1 level was high in normal studied population (Tables 1 and 2). The cause may be due to increased dietary consumption. Aflatoxin B1 was elevated in all subjects having combined HBsAg and anti-HCV antibodies sero-positivity. It was also higher in HBsAg sero-positive than anti-HCV antibodies positive ones with odds ratios = 6.2 and 2.5, respectively (Table 3). These results correspond to those of other studies. [10, 55] It did not constitute an associating factor with bilharzial or fatty liver due to unexplainable factor (Table 4). These results differed from that of Hafez *et al*., (2001) [56] who found aflatoxin B1 in 94.7% of chronic liver disease and 60% in controls with P value less than 0.01. Prevention of aflatoxin B1 adverse effect included reduction in mycotoxin level in food stuffs and further increasing the intake of diet components such as antioxidants and substances known to prevent carcinogenesis.[49] The necessicity to reduce aflatoxin exposure in people living in an area endemic for chronic hepatitis B virus infection was emphasized.[10, 55]

In conclusion, it was found that El Shobak El Sharki population were exposed to environmental pollution by hepatotoxic heavy metals especially cadmium, lead, chromium. It was also, found that anti-HCV antibodies, HBsAg sero-positivities and chronic liver diseases (bilharzial and fatty liver) were associated with hepato-toxic effects of studied

heavy metals. This means that people in this community had multi-exposure risks to more than one factor that could affect the prevalence of liver diseases and the interactions might increase the prevalence of HCC. Trace elements were weakly affected in the studied population. These were zinc level decline in bilharzial liver cases and elevated copper level in bilharzial and fatty liver cases. Also, there was increased in iron level in fatty liver cases. High selenium level could be protective from the sequelae of hepatitis and chronic liver diseases. Chronic liver disease was mostly associated with presence of anti-HCV antibodies. It was also concluded that hepatitis B infection was associated with increase aflatoxin B1 level more than HCV infection. So, prognosis to cirrhosis or HCC was suspected in those having combined HBV and aflatoxin B1. According in the near future the health care system would be confronted by a rush of cases of HCC. It is the time to educate the public about decreasing risk of aflatoxin exposure, and also decreasing the risk of hepatitis B and C exposure. Environmental containment of heavy metals resulting from occupational and non occupational exposure is a must. Also, safe use of insecticides and pesticides must be considered. Early detection of HCC through screening programs is a requirement in the Egyptian community. An intensive study of trace elements, especially iron to evaluate iron stores is also recommended to study host susceptibility factors for contracting hepatitis.

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