Response to Hepatitis B Vaccine in Egyptian Chronic Hepatitis C Patients

Ebada Said[1], Mohamed Metwally[1], Dalia Abd El Hassib[2], Rasha Elsawi[3] and Mohamed Atta[1&4]

Departments of Hepatology, Gastroenterology and Infectious Diseases[1], Benha University, Clinical and Chemical Pathology[2], Benha University, Pathology[3], Benha University and Medicine[4], Rabigh Faculty of Medicine, King Abdulaziz University, Kingdom of Saudi Arabia

Corresponding Author
Ebada Mohamed Said

Mobile:
+201220115116

E mail:
ebadamsaid@yahoo.com

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Background and study aim: Egypt unfortunately has the highest worldwide prevalence of chronic hepatitis C (CHC). Patients with CHC are advised to be vaccinated against hepatitis B virus (HBV) infection. Response to hepatitis B vaccination and risk factors for a weak response are not clearly defined.. The aim of this study is to assess the response to hepatitis B vaccination in CHC patients and identify predictors of a weak response.

Patients and Methods: This prospective study included 112 consecutive adult, treatment- naive, CHC patients (cases group) and 54 non-hepatitis C virus (HCV) subjects (control group). Demographic and laboratory variables including HCV-viral load, schistosomal antibody (Ab) titre, and histopathological examination of liver biopsy were assessed. Three intramuscular 20 µg doses (given at 0, 1 & 6 months) of HBV-vaccine (Euvax-B, Korea) were administered, and hepatitis B surface antibody (HBsAb) titres were evaluated 6 – 8 weeks after the 3rd dose.

Results: Out of 112 CHC patients, five (4.5%) had HBsAb titres of <10 mIU/mL, 20 (17.9%) had <100, and 50 (44.6%) had titres of >1000. In comparison, out of 54 controls, one (1.9%) had a titre of <10 mIU/mL, 2 (3.8%) had <100, and 41 (75.9%) had >1000 (P= 0.001). CHC patients had highly significant lower mean Ab titres than controls (P<0.001). In a univariate regression analysis, HBsAb titre was negatively associated with age (P<0.001), ALT (P=0.03), AST (P=0.03), FIB-4 score (P=0.008) and schistosomal Ab titre (P= 0.007) and positively associated with platelet count (P=0.01). There was no association with gender, BMI, viral load or other variables (including METAVIR grade or stage). A multivariate regression analysis in CHC patients showed that age (P= 0.02) and schistosomal Ab titre (P= 0.04) were independent predictors of HBsAb titre response.

Conclusions: CHC patients, particularly of older age or with schistosomiasis, have a significantly weakened response to the HBV-vaccine.

INTRODUCTION

The most common viral diseases causing chronic liver diseases (CLD) worldwide are hepatitis B virus (HBV) and hepatitis C virus (HCV) infections **[1]**. The global prevalence of hepatitis B surface antigen (HbsAg) positivity varies greatly and studies in the Middle East have shown that Egypt lies in the zone of intermediate prevalence, with HBsAg seroprevalence ranging between 3% to 11%, and genotype D is the most prevalent **[2,3]**. On the other hand, Egypt is cursed with the highest worldwide prevalence of chronic hepatitis C (CHC), with an overall anti-HCV Ab prevalence of 14.7% and it is estimated that 9.8% of Egyptians are chronically infected with HCV. The main genotype in Egypt is genotype 4 (G4), which is responsible for >90% of infections, while the remaining infections are attributable to genotype-1 **[4]**. In patients with dual chronic HCV and HBV infections, disease outcomes, including the development of liver cirrhosis and HCC, are generally more severe than those in patients with monoinfection. In addition, the incidence of HCC in co-infected patients is higher than the incidence in monoinfected patients **[5,6]**.

Both acute and chronic coinfection with HBV in CHC patients is preventable by HBV vaccination **[7,8]**. However, responses to the HBV vaccine are variable among different patients. Response of CHC patients to the HBV vaccine, factors affecting this response, suitable doses and the interval between doses are active areas of research especially in Egypt where HCV-G4 predominates and schistosomiasis is present.

Aim of the study:
To assess the response to HBV- vaccination in CHC patients and identify predictors of a weak response.

PATIENTS AND METHODS

This study was designed as a prospective clinical cohort study to assess the immunogenicity of the HBV vaccine in patients with CHC in comparison to healthy control subjects. The study protocol was approved by the Ethics Committee of Benha Faculty of Medicine. All patients gave written informed consent before enrollment in the study.

One hundred and twelve adult treatment-naive patients with CHC (cases group) and 54 non-HCV subjects (control group) who gave informed consent were included in the study. The inclusion criteria were: age older than 18 years, and CHC (in the cases group) that was diagnosed by both HCV- Ab (by 4th generation ELISA test) and HCV- RNA- PCR positivity for ≥6 months before inclusion in the study. We excluded patients (or controls) who were positive for HBs Ag or HBc Ab (total); underwent previous HBV-vaccination; were pregnant, diabetic; underwent haemodialysis, organ transplantation, or immunosuppressive therapy; or who demonstrated malignancy and/or decompensated cirrhosis with ascites and/or HCC. Non-HCV healthy controls were recruited from subjects who came for vaccination for pre-employment and pre-marital purposes or contacting HbsAg- positive patients.

Demographic data for patients and controls including age, gender and body mass index (BMI) were collected. Laboratory data, including haemoglobin level, white blood cell count, platelet count, liver function profile (ALT, AST, total bilirubin, prothrombin concentration and serum albumin), HCV-viral load and schistosomal Ab titre, were collected for all cases. FIB-4 score was calculated for all cases according to the standard formula **[9,10]**. Abdominal ultrasonography was performed to exclude the presence of ascites and/or hepatic focal lesions, and histopathological data comprising METAVIR necroinflammatory grade (A0-3) and fibrosis stage (F0-4) were reported for those cases who underwent liver biopsy before receiving antiviral treatment within the national project of the Egyptian Ministry of Health.

The Euvax B vaccine (Euvax B, LG Life Sciences, Korea) used in this study is a liquid vaccine consisting of highly purified, non- infectious HBsAg particles absorbed onto aluminium salts as an adjuvant and preserved with thimerosal. It is a recombinant DNA hepatitis B-vaccine derived from HBsAg produced by recombinant DNA technology in yeast cells (Saccharomyces cerevisiae).

Vaccination of both cases and controls was accomplished by administering 3 doses of the Euvax B vaccine, each dose containing 20 µg of the active ingredient, purified HbsAg in a 1-mL volume; the vaccine was intramuscularly injected into the deltoid muscle at 0, 1, and 6 months. The response to the vaccine was measured by quantitatively assessing HBsAb titres (by ELISA test, according to the manufacturer's instructions), 6 - 8 weeks after the 3rd vaccination dose. Non-responders were defined as patients who had an HBs-Ab titre of less than 10 mIU/mL, poor responders were patients with an HBs-Ab titre between 10 and 100 mIU/mL, and good (robust) responders were those who had HBs-Ab titre of more than 100 mIU/mL.

Statistical Methods :
SPSS (version 21) was used for statistical analysis. Comparison of patients and control groups was performed by using a two tailed "**t**" test for continuous variables and a Chi square test for categorical or dichotomous variables. Non-parametric tests were used when indicated. Univariate regression analysis was performed to assess the association between continuous variables and HBs-Ab titre within CHC patients. Independent samples two-tailed "**t**" test was performad to assess the association between categorical or dichotomous variables and HBs-Ab titre. Significant variables associated with HBs-Ab titre in all univariate analyses were included in a multivariate regression analysis to identify independent predictors of the response. Pearson correlation test was performed to test the correlation between age and HBs-Ab titre. For all tests, 0.05 was set as the level of significance.

RESULTS

This study included 112 CHC patients and 54 healthy controls. Out of the 112 patients, 36 (32.1%) were males. Tables (1) and (2) show descriptive demographic, laboratory and histo-pathological data for CHC patients. Only 79 patients had schistosomal- Ab data, and 60 had histopathological data of liver biopsy. Table (3) shows the comparison between cases and control groups in terms of age, gender distribution and BMI. Regarding the response to hepatitis B vaccine, we found that CHC patients had significantly lower HBs-Ab titres than healthy controls and significantly more number of non responders (Table 4 and Figure 1).

Univariate regression analysis showed that there were significant associations between HBs-Ab titre levels and age (P<0.001), AST (P= 0.03), ALT (P= 0.03), FIB4 score (P= 0.008), and platelet count (P= 0.01). Age showed a significantly negative linear correlation with HBs Ab response (Figure 2). There was no statistically significant difference between males and females regarding HBs-Ab titres while there was a statistically significant negative association between the presence of schistosomal Ab and HBs-Ab titre (P= 0.007).

Variables that showed a significant association with HBs-Ab titre in univariate analysis were included in multivariate regression analysis to identify independent predictors of HBs-Ab titre response. The only independent predictors for HBs-Ab response were age and the presence of schistosomal Ab (Table 5).

Table (1): Description of demographic and laboratory data of CHC patients**.**

Parameter	Mean ± SD	Range
Age (Years)	44.2 ± 10.2	**40** (19-59)
Weight (Kg)	82.1 ± 14.4	**81** (45-126)
Height (Meter)	1.62 ± 0.08	**0.35** (1.47-1.82)
BMI	31.5 ± 6.1	**34** (15.2-49.2)
Hb (gm/dL)	13.1 ± 1.5	**8.2** (9.8-18)
WBC (/mm3)	6379 ± 1855	**10300** (2700-13000)
Platelet (×1000)	198.9 ± 62.6	**303** (72-375)
ALT (U/L)	50.9 ± 33	**160** (10-170)
AST (U/L)	47.1 ± 30.2	**193** (12-205)
Bilirubin (mg/dL)	0.87 ± 0.28	**1.35** (0.35-1.7)
Albumin (gm/dL)	4.1 ± 0.39	**2.3** (2.4-4.7)
Proth. Concent. %	89.1 ± 8.1	**40** (60-100)
FIB-4	1.71 ± 1.25	**8.5** (0.34-8.84)

Table (2): Description of schistosomal Ab and histopathology of liver biopsy of CHC patients**.**

Parameter	Number (%)
Schistosomal Ab (79 patients)	
- Positive	**37 (53.2%)**
- Negative	**42 (46.8%)**
METAVIR Necroinflammatory Grade (A) (60 patients)	
A1	**34 (56.7%)**
A2	**24 (40%)**
A3	**2 (3.3%)**
METAVIR fibrosis stage (F)	
F1	**7 (11.7%)**
F2	**28 (46.7%)**
F3	**23 (38.3%)**
F4	**2 (3.3%)**

Table (3): Comparison of demographic variables between cases and controls.

Variables	Cases (N = 112)	Control (N = 54)	P
Gender; **Male**	36 (32.1%)	19 (36.5%)	0.3
Age (years)	44.3±10.3	35.9±7.9	0.05
BMI (kg/m^2)	31.1±7.6	30.8±6.1	0.8

Table (4): Comparison of HBV vaccine responses (HBs Ab titres) between cases and controls.

Variables	Cases (N = 112)	Control (N = 54)	P
HBs-Ab titre (mIU/ml)	675.3 ± 446.5	931.9 ± 337.1	<0.001
Vaccine response			0.001
Non Responders (titer <10 mIU/mL)	5 (4.5%)	1 (1.9%)	
Poor Responders (10-100 mIU/mL)	15 (13.4%)	1 (1.9%)	
Good Responders (>100 mIU/mL)	92 (82.1%)	52 (96.2%)	

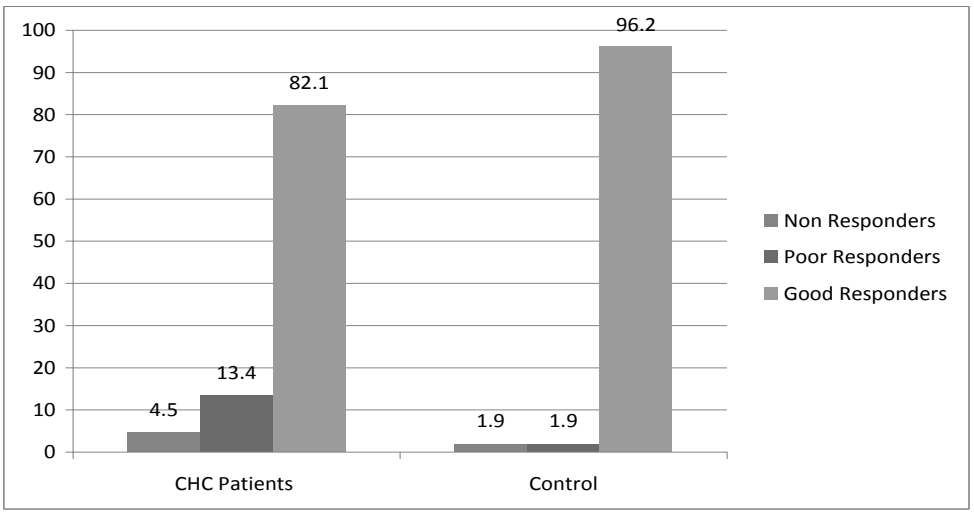

Figure (1): Percentages of non-, poor-, and good- responders among cases and controls.

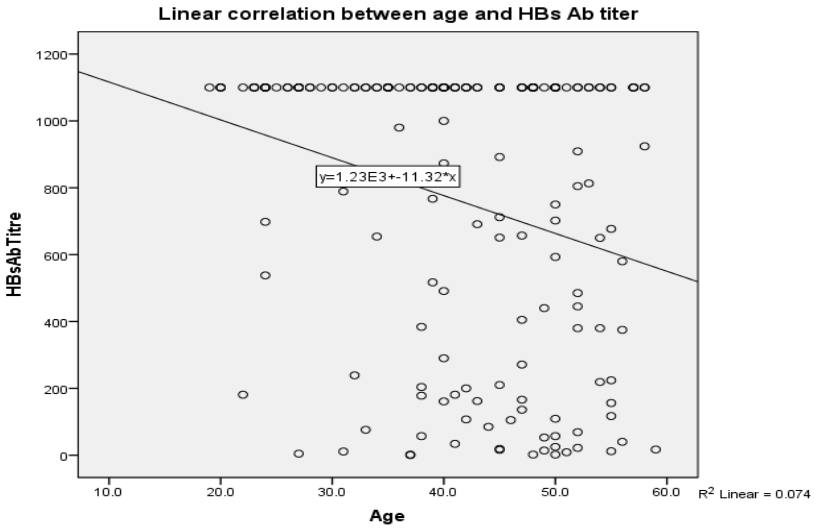

Figure (2): Linear correlation between age and HBs Ab titre..

Table (5): Multivariate regression analysis for independent predictors of the response to HBV vaccination (HBs Ab titre).

Variables	B	95% CI	P
Age (years)	- 15.9	- 29.4 – - 2.6	0.02
Bilharzial Ab	- 227.2	- 438.7 – - 15.7	0.04
BMI (kg/m^2)	31.1±7.6	30.8±6.1	0.8
Platelet count	2.2	- 0.9 – 5.4	0.2
ALT (U/mL)	4.8	- 3.6 – 13.2	0.3
AST (U/mL)	- 8.8	-24.1 – 6.4	0.3
FIB-4 score	155.4	- 163.1 – 473.9	0.3

DISCUSSION

Increased occurrence of fulminant liver failure, rapid progression to cirrhosis and HCC in patients co-infected with HBV and HCV have been documented in many epidemiological studies **[11,12]**. Fortunately, HBV infection is a preventable disease due to the development of HBV-vaccine. However, the vaccination response in patients with CLD is variable.

In the present study, five out of 112 patients with CHC (4.5%) did not respond to HBV- vaccine in comparison to one out of 54 (1.9%) healthy controls. A good (robust) response (HBs Ab >100 mIU/ mL) was achieved in 87/112 (77.6%) cases compared to 51/54 (94.4%) controls. This result is in agreement with a previous study by Minakari et al. **[13]** who found that 4/32 patients with CHC (12.5%) did not respond to HBV vaccination in comparison to 0% of healthy controls. This finding is explained by the evidence that HCV infects immune cells, such as macrophages, B cells, and T cells, with many reports suggesting that the HCV- core, the first protein expressed during the early phase of viral infection, moderates immunomodulatory functions to suppress host immune responses. This altered function of immune cells caused by HCV infection may explain the ineffective immune response to HCV **[14,15]** and may subsequently affect the response to vaccination.

Surprisingly, in Minakari's study, there was an unexplained low but robust response in healthy controls (17/32, 53.1%) compared to the response in 21/32 (65.6%) cases of CHC with genotypes 1 and 3 (in contrast to genotype 4 in >90% of Egyptian cases). Wiedmann et al. **[16]** reported higher failed response rates, observing non-response in 18/59 (31%) of their studied CHC cases compared to 5/58 (9%) healthy controls ($P<0.05$) and low response (anti-HBs 10-99 mIU/mL) in 19% of cases and 17% of controls. The authors were to explain the cause of such a high rate of response failure. On the other hand, Keeffe and Krause **[17]** found that 100% of their studied patients with CHC responded to HBV vaccination. The difference in the seroconversion incidence may be attributed to the difference in the distribution of fibrosis stages or liver disease advancement within each group, as patients with more advanced CLD demonstrate lower response to HBV vaccine **[18,19]**. This phenomenon was also observed in our study, as there was an association between a higher FIB-4 score (reflecting advancement of liver fibrosis) and mean HBs- Ab response level ($P=0.008$).

In a univariate regression analysis, we found that parameters that indicate more fibrosis, such as increased AST levels, decreased platelet counts and increased FIB4 scores, were associated with decreased HBs-Ab titres.

In this study, we found that age was an independent predictor of HBs-Ab response. As age increased, the vaccine response decreased, confirming the concept that older patients demonstrate lower responses to HBV vaccination, as reported by Denis et al. **[20]** who found that only 32 out of 70 patients over 60 years (45.7%) responded to the HBV vaccine, and by de Rave et al. **[21]** who reported a 60% response rate in patients ≥60 years old; this is also in agreement with Al-Zahaby and colleagues **[22]** who recommended a double dose vaccination schedule for such patients.

Schistosomiasis represnts a historical and current health problem in Egypt with estimated prevalence of 3 - 10% in the general population **[23].** In their study of 3,596 Egyptian patients, Abdel-Rahman et al. **[24]** reported that 27.3%

had both HCV-RNA and schistosomal Ab in their sera. Patients demonstrating schistosomiasis/ HCV coinfection have increased HCV morbidity and chronicity **[25]**. Defects in immune response with altered IFN-γ and IL-5 serum levels (related to cell-mediated immunity) and IgE levels (humoral immunity) have been reported, with a relative shift from cellular to humoral immunity, which might play a role in the persistence and severity of both diseases and lower HCV clearance rates **[26,27]**.

The novel finding of our study is the association between the presence of schisosomal Ab and low HBs-Ab titres after vaccination, indicating that schistosomiasis alters the immunologic response to HBV vaccine.

The presence of schistosomal Ab and older age were found to be independent predictors of the response to HBV vaccine in our studied CHC patients.

CONCLUSIONS

Patients with CHC, especially older patients and patients who are schistosomal Ab positive, demonstrate a lower response to HBV vaccination.

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