Clinical Infectious Diseases: Open Access

Elsir et al., Clin Infect Dis 2018, 2:1

Research Article Open Access

Evaluation of Immune Response to Hepatitis B Vaccine in Laboratory Workers, Khartoum, Sudan

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Abstract

This study was conducted to evaluate the responsiveness to hepatitis B virus vaccine in adult healthy laboratory workers in the Faculty of Medical Laboratories Sciences at Al Neelain University, Khartoum, Sudan. The study was conducted at Al Neelain University, during the period from April to June 2014. Sixty one blood samples were collected from healthy adult laboratory workers who received 3 doses of recombinant HBV vaccine. Serum samples were obtained by centrifugation and tested using ELISA to quantify the level of hepatitis B surface antibody in order to detect the responders and non-responders. DNA was extracted from plasma samples collected from the same patients and subjected to polymerase chain reaction (PCR) to detect the presence of Interferon gamma (IFN-γ) A&T alleles in both responders and non-responders.

Out of 61 laboratory workers 54 (88.5%) were found responders and 7 (11.5%) were non-responders to hepatitis B vaccine, with higher frequency of non-responsiveness among the males, there was no association between the responsiveness and age.

All responders and 85% of non-responders were positive for IFN-γ A alleles, while 28.5% of responders and 42.8% of non-responders were positive for IFN-γ T alleles. Responsiveness to hepatitis B surface antigen vaccine is affected by gender (higher in females than males) but not age or the presence of A&T allele of interferon gamma gene.

Keywords: HBV; HCW; ELISA; PCR; IFN-γ; Sudan

Introduction

Hepatitis is defined as an inflammation of the liver, usually caused by viruses, but can be also caused by amoeba, various toxic chemicals, drugs and toxins [1].

Hepatitis-B virus infection is a global public health problem with approximately 400 million people chronically infected [2]. Infection with hepatitis B virus (HBV) leads to a wide spectrum of clinical presentations ranging from an asymptomatic carrier state to self-limited, acute fulminant hepatitis to chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma. Both viral factors as well as the host immune response have been implicated in the pathogenesis and clinical outcome of HBV infection [3]. Despite advances in antiviral therapy, only a minority of chronic hepatitis B patients has a sustained response. Thus primary prevention by vaccination remains the main tool in the control of hepatitis B infection [4].

Sudan is one of the countries with high HBV sero-prevalence in Africa (16%-20% in the general population) [5-7]. Vaccination against HBV is necessary for protecting laboratory workers and other Health care workers. However; not all people respond to the vaccine, a few numbers failed to produce the protective antibodies against HBV after they took the complete doses of the vaccine and thus become at risk of infection with HBV [8,9].

A vaccine for hepatitis B has been available since 1982 and protection is conferred by antibody response to the *a* antigen, an antigen common to all subtypes [10]. Intramuscular vaccine administration at 0, 1, 6 months produces 85-90% seroprotection rate in adolescents [11,12]. When primary vaccination produces HBsAb (hepatitis B surface antibody) level>100 mIU/ml, it is considered to be adequate response (responders). If HBsAb is between 10-100 mIU/ml, then the person is hypo-responder and if it is<10 mIU/ml, then the person is non responder. Hepatitis B surface antibody titre>10 mIU/ml is considered to be a marker of sustained immunity [13]. Seroprotection persists for 10-15 years and so booster vaccination may not be necessary for 15 years post vaccination [14].

Cytokines are components of cell mediated immunity which plays a very important role in the infection. IFN-γ is one of these cytokines; it is so named due to its ability to interfere with viral replication within host cells [15]. Hepatitis B virus (HBV) is susceptible to the cellular immune responses, especially to the signal of IFN-γ. The action of IFN-γ is pleiotropic and causes down regulation of HBV protein, RNA and possibly DNA levels [16].

The present study was conducted to evaluate the responsiveness to hepatitis B vaccine in adult laboratory workers who received hepatitis B vaccine and correlate the responsiveness with the risk factors (age and sex) [17].

Materials and Methods

Study approach

A quantitative approach aimed to evaluate the efficiency of HBV vaccine among vaccinated laboratory workers.

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Received April 02, 2018; **Accepted** April 13, 2018; **Published** April 20, 2018

Citation: Elsir MA, Almoshraf IA, Mustafa MA, Hussein ARME, Elkhidir IM, et al. (2018) Evaluation of Immune Response to Hepatitis B Vaccine in Laboratory Workers, Khartoum, Sudan. Clin Infect Dis 2: 106.

Study area and design

This study was conducted at Al-Neelain University, Department of Medical Microbiology using descriptive, cross-sectional study design.

Study population

The study covered vaccinated laboratory workers at Al-Neelain University, Faculty of Medical Laboratory Sciences, Khartoum, Sudan.

Sample collection

61 Blood samples were collected in plain containers and containers with EDTA for ELISA and DNA extraction respectively after 3 doses of HBV vaccine.

Inclusion and exclusion criteria

All participants that had received complete doses (three doses) of hepatitis B vaccine were included in the study.

Data collection

Data were collected through a structured questionnaire, information on age, gender, numbers and dates of doses, chronic diseases, smoking and place of sample collection, were recorded for each participant.

Data analysis

Collected data were analyzed using the SPSS (statistical package for social science) program. Chi-square statistical analyses were used to determine *P* value significance range.

ELISA test for detecting hepatitis B virus surface antibody (anti-HBs)

Commercial ELISA Kits (Fortress Diagnostic, United Kingdom) were used for this purpose the test was carried out as recommended by the manufacture. In brief, all reagents were allowed to reach room temperature. 50 µl of the calibration curve standards and specimens were added into their respective wells. Then 50 µl of HRP-conjugate reagent was added into each well except the blank. The plate was then covered and incubated for 60 minutes at 37°C. Each well was washed 5 times with diluted wash buffer and 50 µl of chromogen A and B were dispensed into each well including the blank and incubated at 37°C for 15 minutes with light avoidance. 50 µl of stop solution was added into each well. The plate reader was calibrated with the blank well and the absorbance was read at 540 nm.

The results were calculated by subtracting the blank optical density value (OD) from the print report values of samples and control. The absorbance for each calibration standard was then plotted on the Y versus the corresponding anti-HBs concentration on the X on a logarithmic paper and the standard curve was plotted through the plotted points [18].

The concentration of anti-HBs for an unknown was obtained by locating the absorbance (OD) for each unknown on the Y-axis of the graph, find the intersecting point on the standard curve and read the concentration from the X-axis of the graph.

DNA extraction (Saturated sodium chloride method)

300 µl of each EDTA-blood sample was added to 1.5 Eppendorf's tubes. 1000 µl RCLP was added, mixed well and centrifuged at 2500 r.p.m for 10 minutes to obtain clear pellet. Then 300 µl of WCLB and 10 µl of 10% SDS were added to WBC pellet and incubated for 1 hour at room temperature. Then 100 µl of 6 M NaCl and 200 µl of cold chloroform were added and centrifuged at 18000 rpm for 6 minutes. Then the aqueous phase was transferred carefully to other clean Ependorf's tubes to which double volume of cold absolute ethanol was added to precipitate the DNA and centrifuged at 14000 rpm for 5 minutes. The supernatant was poured off without disturbing the precipitate and washed with 600 µl of 70% ethanol. Then the tubes contents were centrifuged at 6000 rpm for 5 minutes, the ethanol was discarded and the tubes were left to air dry. After that each pellet was resuspended in 100 µl TE buffer and left to dissolve overnight [19].

Polymerase chain reaction (PCR) assays

Alleles Specific-PCR was preformed according to Parvaneh [20]. The assay targeted the A/T alleles at the region 260 bp bands correspond to IFN-γ.

The reaction mix with a total volume of 20 µl included 4 µl of master mix solution (5X Hot fire ball) and 2 µl from each specific antisense primers, for A and T, 3 µl of the template DNA and 11 µl of distilled water. The mixture was then gently mixed and then transferred to the PCR machine.

Primers sequences

The primers used were as follows: antisense primer (260 bp corresponded to IFN-γ), TCAACA AAGCTGATACTCCA, sense for T allele sequence: 5'-TTCTTACAACACAAAATCAAA TCT-3´ and sense for A allele sequence: 5´-TTCTTACAACACAAAATCAAATC-3´.

Amplification procedure

Amplification was done in Techne (TC-14) PCR machine. The cycle included initial denaturation at 95°C for 2 min followed by two loops; loop 1 which consisted of 15 cycles with the following program: 95°C for 1 minute, 62°C for 1 minute and 72°C for 1 minute and loop 2 included 25 cycles with the following program: 95°C for 1 minute, 56°C for 1 minute and 72°C for 1 minute for denaturation, annealing and extension respectively and a final extension step at 72°C for 7 minutes [20].

Visualization of products

Absence or presence of PCR products was visualized by electrophoresis (Figure 1). The PCR products were loaded in 1.5% Agarose gel. The gel was prepared as follows: An 0.54 g of the agarose was added to 35 ml of 1X Tris Borate EDAT buffer. The mixture was heated until a homogenous solution was formed, then 0.7 µl of ethedium bromide was added to the mixture [7]. Thirty five ml of the gel were added to the gel box and left to solidify. 8 µl of PCR amplified DNA loaded into the agarose gel wells and 5 µl of the ladder was added to the first well. The gel was then run at 100 V, current 35 A for 30 min and examined by Gel documentation system (INGeNius).

Results

Frequencies of responsiveness to HBV vaccine

Out of 61 heath laboratory workers 54 (88.5%) were found responders and 7 (11.5%) were found non-responders to hepatitis B surface antigen vaccine.

The association between the age groups and the responsiveness to the Hepatitis B vaccine

The participants were categorized into two age groups; among age groups 21-27 years 45/49 (91.8%) responded to the vaccine and among

age group 27-34 years 9/11 (81.8%) responded to the vaccine with insignificant difference (*P* value=0.056) (Table 1).

The association between gender and the responsiveness to the hepatitis B vaccine

Out of 54 responders 12 (22.2%) were males and 42 (93.3%) were females. While out of 7 non-responders 5 (71.4%) were males and 2(28.6%) were females with significant difference between the two genders (*P* value=0.006) (Table 2).

The association between IFN-γ gene A and T alleles and the responsiveness to hepatitis B vaccine

All responders (100%) were positive for IFN-γ A alleles while 85% of non-responders were positive for IFN-γ A alleles. Out of responders 28.5% and 42.8% of non-responders were positive for IFN-γ T alleles respectively.

Discussion

Many reports from different countries described the efficacy of HBV vaccine which generally ranges between 85% to over 90% e.g. Keating and Noble in Switzerland [21] and Desgrand champs and Siegrist in Germany reported sero-conversion rate in HCWs was 95% following 3 doses of recombinant HBV vaccine respectively [22], however this rate was 86% and 86.5% by Zeeshan in Pakistan [23] and by Platkov in Israel [24] respectively. Other epidemiological studies conducted in Iran by Gholamzadeh and Serati, [25] and Saudi Arabia by Zamani [26] showed that the response rate to HBV vaccine equal to 87.3% and 82.8% respectively. However in both studies not all included subjects had completed 3 doses of the vaccine. The authors concluded that, vaccination with the full dose schedule is an important determinant of the response.

Our current study in Sudan is one of the first to report on the responsiveness to HBV vaccine. We found that the rate of the responders was 88.5% and non-responders were 11.5%. This was similar to Chaudhari (2008) in Vasco-Da-Gama, who reported that the rate of responders was 88.4% and of non-responders was 11.4% [27] but different from Mohammed Siddig [28] in Sudan who reported 90% were responders and Zeeshan [23] in Pakistan who reported 86% and 14% for responders and non-responders respectively [29,30].

The study showed that there was no significant association (P value 0.056) with the age which disagreed with the findings by Tripathy [31] and Chaudhari [27], who found that there was strong association between age and response to the vaccine. However, there was significant association to gender with females showing more responsiveness to vaccination [32].

Interestingly, no studies published to report the association between IFN-γ gene A and T alleles and the responsiveness to HBV vaccine. However, Mawada F. Idris reported that the presence of A and T alleles could play a role against hepatitis B infection; changes in the IFN alleles are associated with HBV disease progression and severity of the infection [7].

Finally these findings should highlight the need for the establishment the system to investigate the response to HBV vaccine in Sudan for better management of HBV infections especially in the high risk groups (health care workers, haemodialysis patients and immune compromised patients).

Conclusion

In conclusion, recombinant HBV can be effective in the disease limitation with acceptable responsiveness rate. Therefore; hepatitis B vaccine should be administrated at full doses to all non-vaccinated

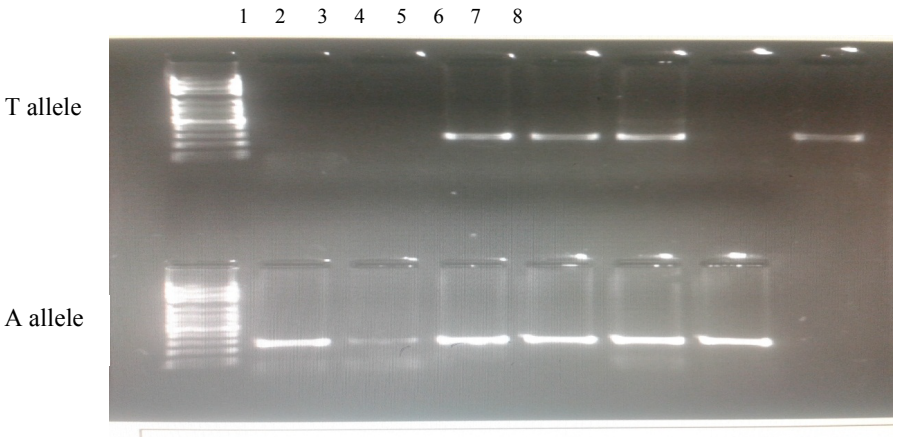

Figure 1: Gel electrophoresis of IFN-γ A and T allele. Lane no. 1 contains 100 bp DNA ladder. Lane no. 2 contains control negative, other lanes contains positive and negative samples for allele T and allele A (Band appears at 260 bp).

Age groups	Responsiveness				Total
	Response		No response		
	No.	%	No.	%	
21-27	48/53	90.6	5/53	9.4	53
28-34	6/8	75.0	2/8	25.0	8
Total	54/61	88.5	7/61	11.5	61

Table 1: The association between the age groups and the responsiveness to hepatitis B vaccine Sudanese laboratory workers 2014.

Responsiveness		Gender		Total
		Males	Females	
Response	No	12/54	42/54	54/61
	%	22.2%	93.3%	88.5%
No response	No	5/7	2/7	7/61
	%	71.4%	28.6%	11.5%

Table 2: The association between gender and the responsiveness to the hepatitis B vaccine in Sudanese laboratory workers 2014.

health care workers. The results obtained should call for wider surveillance at the national level in order to fully elucidate the true status and epidemiology of HBV infection and vaccine response in different areas of Sudan.

Ethical Consideration

The study was approved by the Ethical Review Committee (ERC) of Al-Neelain University, Khartoum State, Sudan. All participants were informed about the aim of the study and asked for their approval before taking the sample. All participants offered an informed consent before being sampled.

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