

Original Research Article

Association Hepatitis B Virus and Non Hodgkin's lymphoma in Sudanese

Patients Sudan

Background: The incidence of non-Hodgkin lymphoma (NHL) is steadily increased worldwide. According to the globocan 2008, NHL is ranked as the 12th most common cancer worldwide and the 4th most common cancer in Sudan. Sudan is endemic with hepatitis B virus (HBV) surface antigen (HBsAg). **Objectives:** The objective of this study was to identify the association between NHL and HBV infection in Sudanese patients. **Methods:** Cross sectional case-control study was conducted at the, National Cancer Institute (NCI) University of Gezira, during 2007 to 2008. HBsAg and HBV DNA was detected in 81 NHL patients and 95 hospital based control matched sex and age using ELISA and polymerase chain reaction (PCR) respectively. The Statistical Program for Social Science (SPSS16.0) was used in performance of all statistical analyses, and test of significant was considered. **Results:** The mean of age of NHL patients was 37.9 ± 23.6 years with rang (1-85years). Male to female ratio is 1.9 to 1. There were different types of NHL in this study the majority were B-cell lymphoma 57/81 (70.4%), Nine out of 81 (11.1%) of NHL patients were positive to HBsAg, while 40/81 (49.38%) were positive by PCR with significant difference between cases and control P-value ($P < 0.05$) there was significant association between B-cell subtype and HBV in both serological and molecular methods (OR= 6.5 95% CI: 1.3 - 32.5) and (OR= 3.6 95% CI: 0.8 – 07.4). **Conclusion:** In conclusion this finding provides one more epidemiologic element supporting the hypothesis that HBV infection may have an association with the development of NHL patients.

Keywords: Non Hodgkin's lymphoma, HBV, DNA, Gezira State, Sudan

1- Introduction

The incidence of non-Hodgkin lymphoma (NHL) is steadily increased during the last several decades. In 2007, approximately over 300,000 newly diagnosed NHL worldwide (Skibola 2007). NHL is ranked as the 12th most common cancer worldwide and the 4th most common cancer in Sudan with age standardize rated (ASR) 5.9 per 100.000 in both sexes (Ferlay et al. 2010). In Sudan NHL is the second cancer in men after prostate cancer with ASR 8.2 per 100.000, while in females rank the fifth with ASR 3.7 per 100.000 (Ferlay et al. 2010). Several studies have reported a significant association between chronic HBV infection and non-Hodgkin's lymphoma (NHL) mainly in HBV endemic areas, while some controversial results on the association in different geographic areas have been reported (Franceschi *et al.*). Notably, an approximately 2-fold increased NHL risk has recently been reported among chronic HBV carriers in a large cohort study from South Korea (Engels *et al.*). Non Hodgkin's Lymphoma comprises a group of lymphoproliferative disorders, the frequency of which continues to rise (Syed et al., 2007). It is possible role for HBV in the induction of B-cell malignant transformation. Moreover, in patients who were diagnosed as hepatocellular carcinomas patients, there was a high detection rate of HBV DNA in peripheral blood lymphocytes (Wang et al., 2007). After HBV infection, both the cell immunity system and the humoral immunity system are activated and exert antiviral effects. The immune system also may destroy the host cells that have been infected by HBV. Thus, the potential role of HBV in B-cell transformation and NHL disease development is probably very complex (Marcucci et al., 2006). Sudan is classified among the African countries with high HBV endemicity (Mahgoub *et al.*). The reported prevalence of HBV chronic infection, characterized by detectable level of HBV surface antigen (HBsAg), varied from region to region and ranged between 5-7% in the general population (Mudawi *et al.*, 2007) and 26% in hospital out-patients (McCarthy *et al.*, 1994). The prevalence of adults having been in contact with HBV and identified by the presence of anti-core antibodies (anti-HBc) was high, ranging between 47.5% and 67% (McCarthy *et al.*, 1994, Mudawi *et al.*, 2007).

Little is known about the risk factors associated with NHL in Sudan. Among environmental factors, the most important is infection especially viral infection. It is useful to conduct an epidemiological study assessing the risk factors for NHL among patients in Central Sudan. This case-control study aimed to identify the association of

HBV and NHL patients attending National Cancer Institute (NCI), University of Gezira.

2- Material and Methods

This study was conducted at the, National Cancer Institute (NCI), Wad Medani, Gezira state, Central Sudan, during 2007 to 2008. It was cross sectional case-control study.

2-1 Study participants

Eighty-one cases (New cases and follow up cases) recruited the inclusion criteria were confirmed cytologically and histopathologically as NHL patients. Patients were examined by ultrasound and bone marrow examination to identify the staging of the disease CT scan and MRI are not routinely used. Diagnostic criteria for determination of sub- type of lymphoma were based on the World Health Organization (WHO)/Revised European-American Lymphoma classification system Ninety five age and sex matched hospital based control were selected. The exclusion criteria were the history of blood transfusions of control. Five ml of venous blood was taken in EDTA after written informed consent after from cases and controls. Personal data and disease information was gathered using a questionnaire. It included age, sex, occupation, residence, past medical history, history of blood transfusion. Data of diagnosis, type of NHL (T, B), and stage of NHL was collected from patient notes. Ethical approval was obtained from National Cancer Institute Research Ethical Committee (NCI-ERC).

2.2- Detection of HBV

2.2.1- Detection of HBsAg

All serum samples was detected for HBsAg HBV surface antigen (HBsAg) was detected by screening test using HBsAg ELISA Kit (Monolisa™ HBs Ag ULTRA, Marnes-la-Coquette-France) according to manufacture instructions

2.2-II- Molecular Detection of HBV Infection

Viral DNA was extracted from the study participant's plasma samples using cinnaGen IncDNP™ Kit (DN 8115C) Tehran, IRAN. HBV was detected by PCR using cinnaGen IncDNP™ PCR Kit as recommended by the manufacturer. Positive samples were identified by presence of a 353 bp PCR product by electrophoresis using 2% agarose gel (figure 1).

2.3- Statistical analysis

Data were expressed as mean and standard deviation (SD) and, as appropriate. Comparison of HBsAg and HBV DNA prevalence between the NHL group and the control group was presented as adjusted odds ratio and 95% confidence intervals. Comparative analyses of clinico- pathologic (NHL Types and Stages) characteristics between the two groups were performed using the Chi-square test, and Fisher's exact test as appropriate. The Statistical Program for Social Science (SPSS16.0) was used in performance of all statistical analyses, and p-value less than 0.05 was considered significant.

3- Results and Discussion

3.1- Study participants

The study consisted of a total of 81 NHL cases and 95 hospital-based controls matched age and sex $\pm$ 5 years. The mean of age of NHL cases and control were shown in table 1. Male to female ratio of NHL cases were 2 to 1. There were different types of NHL in this study the majority were B-cell lymphoma 57/81 (70.4%), while Burkitt's lymphoma represent 14/81 (17.3%); T-cell lymphoma 7/81 (8.6%) as in table 1.

3.1- NHL and HBV association

3.1. i) HBV infection

Serological test was performed for HBsAg 9/81(11.1%) cases were HBsAg positive while only 2 (2.1%) in controls, there was a significant different between cases and control $P=0.03$ Odd ratio (OR) (CI95%) 5.8(1.2-27.7). HBV DNA was found in 40/81 (49.38%) of NHL cases and 20/95 (21.05%) of controls. This result showed significant and highly significant difference between cases and controls $P= 0.0001$ (OR) (CI95%) 3.7 (1.9-7.1) as in table 1.

3.1.ii) HBV infection as risk factors for and NHL

B-cell subtype is the most common type of NHL in this study. There was significant association between Non Hodgkin's lymphoma and HBV in both serological and molecular methods (OR= 6.5 95% CI: 1.3 - 32.5) and (OR= 3.6 95% CI: 0.8 – 07.4). No association was found between the other NHL subtypes and HBV with both methods (Sample size was small) table 2.

Different NHL stages were found in this study According to the WHO classification, stage IIIB was the most common stage 24 (29.6%). There were significant association between stages IVA stage, $P= 0.001$ OR (CI95%) 22.5 (2.5 -197.8) and IEA stage,

OR (CI95%) 18.7 (2.1-169.7) and HBV-DNA, while no significant association between IEA stage HBV infection serological method as in table 3.

3.2. Discussion

The incidence of Non-Hodgkin lymphoma (NHL) has been rising steadily for the past 30 years, but has leveled off in recent years (Spinelli et al. 2008) Lymphomagenesis is a multifactorial process in which genetic, environmental, and infectious factors can be involved. In this study only HBV was selected, other than these viruses because it more common and Sudan was classified as an endemic country of HBV (Mudawi et al. 2007) . Lymphoid cancers are more frequent in males than females throughout all age groups (Muller et al. 2005). In this study male to female ratio is 1.9:1 and thus is in line with previous observations. HBsAg was detected without an evidence of chronic HBV infection, while HBV DNA was detected in the serum using conventional PCR. HBV DNA was detectable in the serum of persons with acute and chronic HBV infection (Rotman et al. 2009). The result showed a highly significant difference between cases and control by serology and HBV-DNA infection ($P= 0.03$ and 0.0001 respectively). (The odd ratio OR = 3.7-5.6). Nath and his colleagues in Saudi Arabia found that in case control study an association between HBV and NHL (odds ratios 1.5–3.6) (Nath et al.)2006 . In Egypt Alsayed et al found that HBsAg positivity was not different in NHL patients when compared to controls, yet it was significantly more encountered in LN of NHL patients ($p = 0.04$). HBV-DNA was detected in patient's samples and none of the controls (El-Sayed et al. 2006).

In South Korea among NHL subtypes, HBsAg positivity was associated with increased risk of diffuse large B-cell lymphoma (Engels et al.)2011, (Franceschi et al.) 2011 found a positive association between HBsAg seropositivity and the risk of NHL but was not statistically significant. Our findings showed there was significant association between B-cell lymphoma and HBV infection HBsAg and HBV- DNA (OR= 6.5 95%CI: 1.3 -32.5) (OR= 3.6 95%CI: 0.8 – 07.4) respectively. A case-control study performed in Korea by Kim *et al.*, (2002), they found that there was a significant difference ($P = 0.001$) in HBV infection and non-Hodgkin's lymphoma cases compared to control (Kim *et al.*, 2002) (Kim *et al.*)2011 mentioned high HBV prevalence was observed not only in B-cell NHL but also in the T-cell NHL group. that HBV may play a significant role in development of NHL, particularly in HBV endemic areas. Park and colleagues stated that in NHL patients from Korea the prevalence of HBV infection was higher in B-cell non-Hodgkin's lymphoma than

control (Park et al. 2008). These results were consistent with our finding in B cell subtype. This difference in results might be due to regional variation, the prevalence of chronic HBV infection varies widely in different parts of the world. The evidence from these studies, showing the presence of HBV infection more than a decade before NHL diagnosis, suggests that HBV might have a causal role in the development of NHL.

The present study has strengths and weaknesses. The study identifies the association between HBV and NHL in Sudanese. Because various NHL subtypes the study samples were small and some subtypes were not well tested. Unfortunately, we could not include additional serum markers (eg, HBeAg, HBcAg) that would show severity and chronicity of HBV infection also HBV DNA was detected by PCR conventional method not RT-PCR (viral load) due to the fund limitations. Our results are unlikely to be affected by confounding by infection with HCV or HIV which were screened in this study.

In conclusion, there is an association between NHL and HBV infection in Sudanese. HBV infection was more common in B cell NHL. This finding provides one more epidemiologic element supporting the hypothesis that HBV infection may have an association with the development of NHL in Sudanese. The recommendation of this study more samples and studies were needed to identify the genotypes of HBV in NHL patients and the mechanism of carcinogenesis of HBV and NHL in Sudanese.

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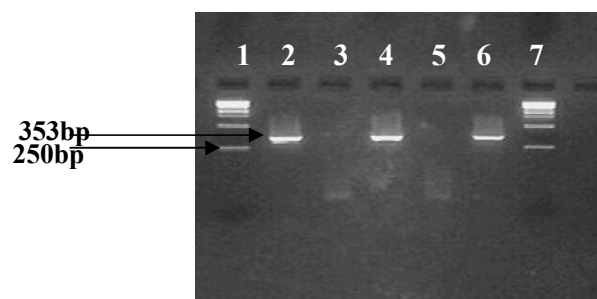


Figure: 1. Lane 1, 7 DNA ladder, Lane 2: positive control, Lane 3: negative control, Lane 4,6 positive samples, Lane 5: negative sample

Table 1 shows the association between NHL and HBV infection by serological and Molecular methods

	NHL group 81	Control group 95	<i>p</i> -Value	OR (95%CI)
Total No. of Subjects				
Males (%)	53 (65.4)	65 (68.4)		
Age (mean $\pm$ SD)	(36.63 $\pm$ 25.56)	(31.01 $\pm$ 22.12)		
Females (%)	28 (34.6)	30 (31.6)		
Age (mean $\pm$ SD)	(40.39 $\pm$ 19.66)	(37.30 $\pm$ 17.09)		
Total HBsAg positive (%)	9 (11.11)	2 (2.10)		
In males	6 (66.7)	2 (100)	0.03*	5.8 (1.2 -27.7)
In females	3 (33.3)	0		
Total HBV-DNA positive (%)	40(49.4)	20 (21.1)		
In males	28 (70)	14 (70)	0.0001**	3.7 (1.9 – 7.1)
In females	12 (30)	6 (30)		

*Significant association HBV and NHL

**Highly Significant association HBV and NHL

Table 2 shows the association between NHL and HBV infection compared with different types of NHL

Total No. of Subjects	NHL group 81	Control group 95	<i>p</i> -Value	OR (95%CI)
B-Cell NHL				
No of Subjects (%)	57 (70.4)	95		
Age (mean ± SD)	(42.4 ± 2.3 E1)	(33.0 ± 2.1 E1)		
HBsAg positive (%)	7 (12.5)	2 (2.10)	0.01*	6.5 (1.3 - 32.5)
HBV-DNA positive (%)	28 (49.1)	20 (21.1)	0.0001**	3.6 (0.8 – 07.4)
Burkitt's lymphoma				
No of Subjects (%)	14 (17.3)	95		
Age (mean ± SD)	(13.9 ± 1.2 E1)	(33.0 ± 2.1 E1)		
HBsAg positive (%)	0 (0)	2 (2.10)	-	-
HBV-DNA positive (%)	6(42.9)	20 (21.1)	0.07	2.8 (0.9 – 9.0)
T-Cell NHL				
No of Subjects (%)	7 (8.6)	95		
Age (mean ± SD)	(43.4 ± 2.3 E1)	(33.0 ± 2.1 E1)		
HBsAg positive (%)	1 (14.4)	2 (2.10)	0.2	7.7 (0.6 – 98.1)
HBV-DNA positive (%)	4 (57.1)	20 (21.1)	0.05	5.0 (1.0 – 24.2)
unknown				
No of Subjects (%)	3(3.7)	95		
Age (mean ± SD)	(52.3 ± 2.8 E1)	(33.0 ± 2.1 E1)		
HBsAg positive (%)	1(33.3)	2 (2.10)	-	-
HBV-DNA positive (%)	2(66.7)	20 (21.1)	-	-

*Significant association HBV and NHL

**Highly Significant association HBV and NHL

Table 3 show the comparison of the different stages of NHL and HBV in the study subject

	NHL group	Control group	p-Value	OR (95%CI)
Total No. of Subjects	81	95		
HBsAg positive (%)	9 (11.1)	2 (2.1)	0.03	5.8 (1.2 -27.7)
HBV- DNA positive (%)	40(49.4)	20 (21.1)	0.0001	3.7 (1.9 – 7.1)
Stage IIB				
No of Subjects (%)	7(8.6)	95		
HBsAg positive (%)	0	2 (2.1)	-	-
HBV- DNA positive (%)	1 (2.5)	20 (21.1)	0.5	1.6 (0.2-14.1)
Stage IB				
No of Subjects (%)	1 (1.2)	95		
HBsAg positive (%)	1 (100)	2 (2.1)	-	-
HBV- DNA positive (%)	1 (100)	20 (21.1)	0.2	-
Stage IVB				
No of Subjects (%)	13 (16.6)	95		
HBsAg positive (%)	1 (7.7)	2 (2.1)		
HBV- DNA positive (%)	1 (7.7)	20 (21.1)	0.2	0.3 (0.03-2.5)
StageI IIIB				
No of Subjects (%)	24 (29.6)	95		
HBsAg positive (%)	3 (12.5)	2 (2.1)	0.05	6.6 (1.0 -42.2)
HBV- DNA positive (%)	8 (33.3)	20 (21.1)	0.2	1.9 (0.7-5.0)
Stage IVA				
No of Subjects (%)	7(8.6)	95		18.6 (2.15 -
HBsAg positive (%)	2(28.6)	2 (2.1)	0.02	160.7)
HBV- DNA positive (%)	6(85.7)	20 (21.1)	0.0001*	22.5 (2.5 -197.8)
Stage IEA				
No of Subjects (%)	6 (7.4)	95		
HBsAg positive (%)	1(16.7)	2 (2.1)	0.1	7.7 (0.6 -98.1)
HBV- DNA positive (%)	5 (83.3)	20 (21.1)	0.001*	18.7 (2.1-169.7)
Stage IIIA				
No of Subjects (%)	12 (14.8)	95		
HBsAg positive (%)	1(8.3)	2 (2.1)	0.3	4.2(0.3 -50.5)
HBV- DNA positive (%)	4 (33.3)	20 (21.1)	0.3	1.9 (0.5-6.9)
Stage IIIEA				
No of Subjects (%)	1 (1.2)	95		
HBsAg positive (%)	0	2 (2.1)	-	-
HBV- DNA positive (%)	1 (2.5)	20 (21.1)	0.2	-
Stage IA				
No of Subjects (%)	10 (12.3)	95		
HBsAg positive (%)	0	2 (2.1)	-	-
HBV- DNA positive (%)	4 (10.0)	20 (21.1)	0.2	2.5 (0.6 -9.7)

*Significant association HBV and NHL