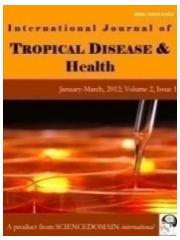

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The Association between Hepatitis B Virus and Non Hodgkin's Lymphoma in Sudanese Patients in Gezira State Sudan

Nagla Gasmelseed[1*], Afrah Awad Elsir[1] and Ahmed Elhaj Mohamed[2]

[1]Department of Molecular Biology, National Cancer Institute, Sudan.
[2]Department of Oncology, National Cancer Institute Nagla Gasmelseed, Department of Molecular Biology, National Cancer Institute University of Gezira, Wad Medani, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The incidence of non-Hodgkin lymphoma (NHL) is steadily increasing 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) infection.

Objectives: The objective of this study was to identify the association between NHL and HBV infection in Sudanese patients, Gezira State.

Methods: This was cross sectional case-control study was conducted at the, National Cancer Institute (NCI) University of Gezira, during the period from 2007 to 2008. A total of 81 non-Hodgkin lymphoma patients and 95 hospitals based controlled of matched age and sex, were included in the study. All patients and control had their serum screened for the presence of HBsAg by ELISA and HBV DNA was tested using polymerase chain reaction (PCR). The Statistical Program for Social Science (SPSS16.0) was used for all statistical analyses, and test of significance.

Results: The mean age of NHL patients was 37.9±23.6 years with a range (1-85 years). Male to female ratio was 1.9 to 1. There were different types of NHL in this study with the majority being B-cell lymphoma 70.4% (57/81), 49.38% (40/81) were positive by PCR,

**Corresponding author: Email: nag_la@yahoo.com;*

while controls 20/95 (21.05%) with significant difference between cases and controls (P<0.05). There was a significant association between B-cell subtype and HBV by 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: This study concludes that there is a significant association between HBV and the development of NHL in Sudanese patients in Gezira State the finding provides evidence 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 increasing during the last several decades. In 2007, there were approximately over 300,000 newly diagnosed NHL worldwide [1]. NHL is ranked as the 12th most common cancer worldwide and the 4th most common cancer in Sudan with an age standardized rate (ASR) of 5.9 per 100.000 in both sexes [2]. In Sudan NHL is the second most common cancer in men after prostate cancer with an ASR of 8.2 per 100.000, while in females rank the fifth with ASR of 3.7 per 100.000 [2]. Several studies have reported a significant association between chronic HBV infection and non-Hodgkin's lymphoma (NHL) mainly in HBV endemic areas. on other hand some controversial results on the association in different geographic areas have been reported [3]. Notably, an approximately 2-fold increased NHL risk has recently been reported among chronic HBV carriers in a large cohort study from South Korea [4]. Non-Hodgkin's Lymphoma comprises a group of lymphoproliferative disorders, the frequency of which continues to rise [5]. There is a possible role for HBV in the induction of B-cell malignant transformation. Moreover, in patients who were diagnosed as hepatocellular carcinomas, there was a high detection rate of HBV DNA in the peripheral blood lymphocytes [6]. After HBV infection, both the cellular and the humoral immunity systems 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 [7]. Sudan is classified among the African countries with high HBV endemicity [8]. The reported prevalence of HBV chronic infection, based on detectable levels of HBV surface antigen (HBsAg), varies from one region to another and ranging between 5-7% in the general population [9] and up to 26% in hospital out-patients (McCarthy et al. [10]). The prevalence of adults having been in contact with HBV and identified by the presence of anti-core antibodies (anti-HBc) is high, ranging between 47.5% and 67% [10,11].

Little is known about the risk factors associated with NHL in Sudan. Among environmental factors, the most important is viral infection. This case-control study aims to identify the association of HBV and NHL in patients attending National Cancer Institute (NCI), University of Gezira.

2. MATERIALS AND METHODS

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

2.1 Study Participants

Eighty-one cases (New cases and followed up of non-Hodgkin lymphoma (NHL) patients, who were attended National Cancer Institute and confirmed cytologically and histopathologically were included in the study. Patients were examined by ultrasound and bone marrow examination to identify the staging of the disease. 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 of age and sex matched hospital based control were then selected. Controls with a positive history of blood transfusion were excluded from the study. Five ml of venous blood were collected in EDTA- containing tubes after obtaining a written informed consent from both cases and controls. Personal and clinical were collected using a questionnaire. It included age, sex, occupation, residence, past medical history and history of blood transfusion. Data of diagnosis, types of NHL (T, B) and stages of NHL were collected from patient notes. Ethical approval was obtained from the National Cancer Institute Research Ethical Committee (NCI-ERC).

2.2 Detection of HBV

2.2.1 Serological detection of HBsAg

All serum samples were screened for HBsAg HBV surface antigen (HBsAg), by using HBsAg ELISA Kit (Monolisa™ HBs Ag ULTRA, Marnes-la-Coquette-France) according to the manufacture instructions.

2.2.2 Molecular detection of HBV infection

Viral DNA was extracted from the study participant's plasma samples by PCR using cinnaGen IncDNP™ Kit (DN 8115C) Tehran, IRAN. HBV was detected by PCR using cinnaGen IncDNP™ PCR Kit as recommended by the manufacturer. The total volume of PCR reaction was 25µl with 10 µl DNA. PCR amplification was done in steps as shown in Table 1. Positive samples were identified by the presence of a 353 bp PCR product by electrophoresis using 2% agarose gel (Fig. 1).

Table 1. PCR protocol

Steps	Temperature - time	Cycle
Initiation	93ºC-60 sec 61ºC-20 sec	1
Annealing	72ºC -40 sec	
Elongation	93ºC-20 sec 61ºC -20 sec 72ºC-40 sec	35
End	4ºC	

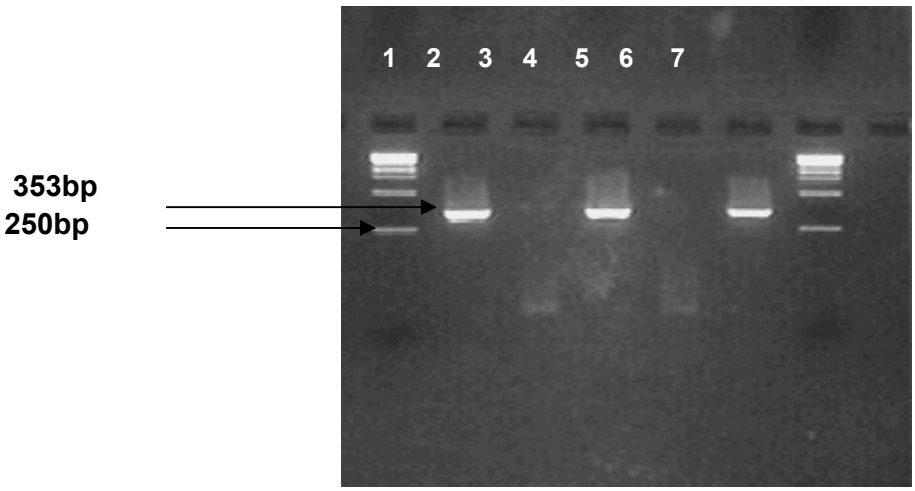

Fig. 1. Lane 1, 7 DNA ladder, Lane 2: positive control, Lane 3: negative control, Lane 4,6 positive samples, Lane 5: negative sample

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 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

3.1 Study Participants

The study consisted of a total of 81 NHL cases and 95 hospital-based controls of matched age and sex ± 5 years. The mean age of NHL cases and control were shown in Table 1. Male to female ratio of NHL cases was 2:1. There were different types of NHL in this study, the majority were B-cell lymphoma 70.4% (57/81), followed by Burkitt's lymphoma that represented 17.3% (14/81) and then T-cell lymphoma 818.6% (7/) as in Table 3.

3.2 NHL and HBV Association

3.2.1 HBV infection

When the serological test was performed for HBsAg in NHL patients, 11.1% 9 cases were HBsAg positive while only 2 (2.1%) were positive among controls. There was a significance 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 a highly significant difference between cases and controls P= 0.0001 (OR) (CI95%) 3.7 (1.9-7.1) as in Table 2.

Table 2. Shows the association between NHL and HBV infection by serological and Molecular methods

	NHL group	Control group	*p*-value	OR (95%CI)
Total no. of subjects	81	95		
Males (%)	53 (65.4)	65 (68.4)		
Age (mean ± SD)	(36.63±25.56)	(31.01±22.12)		
Females (%)	28 (34.6)	30 (31.6)		
Age (mean ± SD)	(40.39±19.66)	(37.30±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; SD: Standard deviation, P: Probability, OR: Odd Ratio, CI: Confidence Interval*

3.2.2 HBV infection as a risk factor for and NHL

B-cell subtype is the most common type of NHL in this study. There was a 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 Table 3.

Table 3. Shows the association between NHL and HBV infection compared with different types of NHL

	NHL group	Control group	*p*-value	OR (95%CI)
Total no. of subjects	81	95		
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 SD: Standard deviation, P: probability, OR: Odd Ratio, CI: Confidence Interval*

Different NHL stages were found in this study According to the WHO classification, stage IIIB was the most common stage 29.6% (24). There was significant association between stages:

IVA stage, (P= 0.001) Odd ratio (OR) with confidence interval 95% (CI95%) 22.5 (2.5 -197.8) IEA stage, OR (CI95%) 18.7 (2.1-169.7) and HBV-DNA. No significant association was found between stage IEA and HBV infection according to serological methods as in Table 4.

Table 4. 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 -160.7)
HBsAg positive (%)	2(28.6)	2 (2.1)	0.02	22.5 (2.5 -197.8)
HBV- DNA positive (%)	6(85.7)	20 (21.1)	0.0001*	
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; SD: Standard deviation P: probability: OR : Odd Ratio CI : Confidence Interval*

4. DISCUSSION

The incidence of Non-Hodgkin lymphoma (NHL) has been rising steadily for the last 30 years, but has leveled off in recent years [12]. Lymphomagenesis is a multifactorial process in which genetic, environmental and infectious factors can be involved. In this study HBV

was selected, than other viruses because it more common in Sudan, which is classified as an endemic country for HBV [12]. Lymphomas are more frequent in males than in females throughout all age groups [13]. In this study male to female ratio was 1.9:1 and thus it is keeping in the line with the previous observations. HBsAg was detected without clinical evidence of chronic HBV infection, while HBV DNA was detected in the serum using conventional PCR. This was consistent with the results of Roman et al, who proved the presence of HBV DNA in the serum of persons with both acute and chronic HBV infection [14]. The results showed a highly significant difference between cases and controls according to serology and PCR with respect to HBV-DNA testing (*P*= 0.03 and 0.0001 respectively), (The odd ratio OR = 3.7-5.6). A case control study conducted in Saudi Arabia has found an association between HBV and NHL (odds ratios 1·5–3·6) [15]. In Egypt Alsyed et al found that HBsAg positivity was not different in NHL patients when compared to controls, yet it was significantly more encountered in lymph nodes of NHL patients (p = 0.04). HBV-DNA was detected in patient's samples and none of the controls [5].

In South Korea among NHL patients, HBsAg positivity was associated with increased risk of diffuse large B-cell lymphoma [4,3]. There was a positive association between HBsAg seropositivity and the risk of NHL, which was not statistically significant. Our findings showed there was a significant association between B-cell lymphoma, HBsAg and HBV- DNA (OR= 6.5 95%CI: 1.3 -32.5), (OR= 3.6 95%CI: 0.8 – 07.4) respectively. In a case-control study performed in Korea by Kim et al. showed that there was a significant difference (P = 0.001) in HBV infection and non-Hodgkin's lymphoma cases compared to controls [16] Kim et al. noticed a high HBV prevalence not only in B-cell NHL but also in the T-cell NHL group. Never the less HBV may play a significant role in the 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 among controls [17]. These results were consistent with our findings in B cell subtype. This difference in results might be due to regional variations, as 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 in more than a decade before NHL diagnosis, suggests that HBV might have a crucial role in the development of NHL.

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

5. CONCLUSION

In conclusion, there is a significant association between NHL and HBV infection in Sudanese patients in Gezira State. This finding provides evidence, supporting the hypothesis that HBV infection may have an association with the development of NHL in Sudanese patients.

It is recommended that more studies containing large samples size are needed to identify the genotypes of HBV in NHL patients and the mechanism of carcinogenesis of HBV and NHL in Sudanese patients.

CONSENT

It is applicable written informed consent was taken from each patients included in this study.

ETHICAL APPROVAL

Ethical approval has been taken from National Cancer Institute Ethical committee before conducting this study

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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