MEDICAL CHANNEL

Original Article

FREQUENCY OF HEPATITIS B VIRUS (HBV) INFECTION IN PATIENTS OF NON-HODGKIN'S LYMPHOMA (NHL) AT TERTIARY CARE CENTERS OF LARKANA.

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ABSTRACT:

Objective: To determine the frequency of hepatitis B virus (HBV) infection in patients of Non- Hodgkin's Lymphoma (NHL) at tertiary care centers of Larkana.
Study Design: This is a Cross sectional study.
Study Setting: This study was conducted at Department of Medicine in collaboration with Department of Pathology, Chandka Medical College (C.M.C) Hospital Larkana and Larkana Institute of Nuclear Medicine and Radiotherapy (LINAR), over two years period from March 2008 to February 2010.
Material and Method: Total 127 cases of biopsy proven non-Hodgkin's lymphoma, age 5-74 years of either sex visited medical OPD of CMC Teaching Hospital Larkana and LINAR were included, blood samples were taken from participants for detection of hepatitis B surface antigen.
Results: Out of 127 cases, male were 78 (61.4%) and female were 49 (38.6%) among them 26 (20.4%) patients were infected by hepatitis B virus. Infected male were 15 (57.7%) and female were 11 (42.3%). The maximum infected patients were in the 41-60 years of age grouping i.e. 11 (42.3%). High grade and stages-1V of NHL patients were infected mostly, 14 (53.8%) and 11(42.3%) respectively. B symptoms were observed in 17 (65.38%) out of 26 patients of infected group in comparison to 60 (59.40%) of non-infected group.
Conclusion: This study shows high frequency of hepatitis B virus infection in Non-Hodgkin's lymphoma patients, particularly in higher grade and stage.

Key Words: Hepatitis B Virus, Non-Hodgkin's Lymphoma, High Grade, Stage 1V.

MATERIAL AND METHODS

This cross-sectional study was conducted at Department of Medicine in collaboration with Department of Pathology, Chandka Medical College (C.M.C) Hospital Larkana and Larkana Institute of Nuclear Medicine and Radiotherapy (LINAR). Over a period of 2 years from March 2008 to February 2010.

For this study, approval of ethical committee of hospital was taken, then 127 consecutive, histologically proven patients of NHL (any grade or stage), age 5-74 years, of either sex visited medical OPD of CMC Teaching Hospital Larkana and LINAR, were included, after obtaining their consent by principal researcher.

Blood samples were taken from participants, coded and sent to C.M.C Hospital Laboratory (Department of Pathology) for detection of anti-HBsAg antibodies on 2nd generation ELISA (Enzyme-Linked Immunosorbent Assay), Human Immunodeficiency Virus (HIV) and Hepatitis D Virus antibodies. NHL grade was noted from biopsy report. Ultrasound and computerized tomography scan of chest and abdomen was performed to confirm the stage of NHL. Data including the variables, age, gender, and stage of NHL, and presence or absence of HBV was collected and transferred to a self-designed performa by principal researcher.

Data Analysis Procedure: The collected data was analyzed using computer software SPSS 17.0 version. Descriptive statistics were used to summarize categorical and continuous

data accordingly as Gender, presence or absence of HBV, Grading of NHL and Staging of NHL and was presented in percentages and proportions for data presentation.

Exclusion Criteria: Any patient above the age of 75, non consenting or previous diagnosed HBV patients, Patients with past exposure of radiation or chemotherapy and receiving palliative or curative therapy for NHL, Patients suffering from Human Immunodeficiency Virus (HIV) infection, HBV with HDV, immunodeficiency states and autoimmune disorders were excluded, patients who had received or under gone any blood transfusion and major surgical procedure in last six months or previously diagnosed as a case of medical disorder requiring parenteral medications e.g. Hemophilia, and thalessemia, etc were also excluded.

INTRODUCTION:

Hepatitis B virus is a 42 nm double stranded, encapsulated DNA (deoxyribonucleic acid) virus, and is the prototype of hepadnavirus family. There are eight genotypes (A-H) and sub types. In Pakistan, genotype D is most prevalent.

Worldwide there are nearly two billion people infected with hepatitis B virus, more than 400 million[1] have chronic liver disease, and >350 million peoples are chronic HBsAg carriers. The frequency of carrier rate varies from 1% in United States and Western Europe to 35 % in parts of Africa and Asia. In the United States 1.25 million (predominantly males)[1] peoples are infected with hepatitis B virus. An estimated 600000 persons die each year due to the acute or chronic consequences of hepatitis B. [2] It is a tenth leading cause of death worldwide. Given its large population (170 million) and intermediate to high rates of infection, Pakistan is among the worst afflicted nations. Pakistan Medical and Research Council (PMRC) survey shows 7.4% (12 million) of Pakistani peoples are infected with HBV and (Hepatitis C Virus) HCV.[3] The prevalence of HBV in Pakistan range from 2.11% to 10% [4,5] with marked difference in all four provinces. Recent survey reports shows prevalence for hepatitis B virus is 2.5% in healthy population of Pakistan. In province of Sindh, prevalence of HBV is 2.5%, and in Larkana, prevalence is 4.3%.[3]

Hepatitis B infection is associated with various extra hepatic manifestations [6,7] i.e.

glomrulonephritis[8], mixed cryo globulinemia[9], polarteritis nodosa[10], serum sickness like syndrome, arthritis, glucose intolerance and diabetes mellitus[11], porphyria cutanea tarda, peripheral neuropath, myeloproliferative disorders. Hepatitis B virus is also lymphotropic[12], so it can predispose to lymph proliferative disorders as NHL (Non Hodgkin Lymphoma). NHL has steadily increased over past several decades and so is the epidemic of HBV infection in rural areas of Pakistan. Worldwide various studies prove high frequency of hepatitis B virus in non-Hodgkin's lymphoma in compare to their general population and or control group.[13-20]

Lymphoma is a group of cancers that affect the cells (B and T lymphocytes) that play a role in the immune system and primarily represents cells involved in the lymph nodes and other lymphoid tissues (such as the spleen and bone marrow). In lymphoma, there is malignant transformation of either B or T cells or their subtypes. Lymphoma is the most common type of blood cancer in the United States. They are the seventh most common cancer in adults and the third most common in children. There are two main types of lymphomas. Hodgkin lymphoma and Non-Hodgkin's lymphomas. Non-Hodgkin's lymphomas are far more common than Hodgkin's lymphoma.

Non-Hodgkin's lymphoma (NHL) is a heterogeneous disease, includes all types of lymphoma except Hodgkin lymphoma. Each year, there are approximately 66,000 new cases [21, 22] and almost 20,000 deaths from NHL in the United States.[21, 22] Non-Hodgkin's lymphoma occurs more often in patients between the ages of 40 and 70 but it can occur at any age. Incidence of NHL increases as the age advance i.e. 2.4/100000 at age of 20- 40 years, 46.2/ 100000 at the age of 60-64 years, and >100/100000 after age of 75 years.[23]

Number of factors have been associated with an increased risk for NHL, including congenital and acquired immunodeficiency states, physical, and chemical agents, Infectious agents, such as viral infections (e.g., Epstein Barr virus, HIV, human T-cell leukemia virus), and bacterial infections (e.g. Helicobacter Pylori) may be associated with the development of NHL. Additionally, physical and chemical agents such as pesticides, solvents, arsenate, lead as well as hair dyes, radiation exposure (high dose), and paint thinners may increase the risk.

NHL is comprised of approximately 10 different subtypes in the Working Formulation and 20 different disease entities in the Revised European-American Lymphoma Classification (REAL) system.[24] These subtypes are grouped into three biologic states- low grade, intermediate grade, and high-grade lymphoma

Recently World Health Organization (WHO) [25] has classified the lymphoma based on how they look under a microscope, the chromosome features of the lymphoma cells, and the presence of certain chemicals on the surface of the cells. B-cell NHL makes up most (about 85%) of non-Hodgkin lymphomas in the United States. Diffuse large B-cell lymphoma, Follicular lymphoma are two most common types. While T cell accounts for 15% of NHL. NHL often presents with painless swelling (Lymph adenopathy) in the neck, under an arm, or in the groin. The enlarged lymph node sometimes causes other symptoms by pressing against a vein or lymphatic vessel (swelling of an arm or leg), a nerve (pain, numbness, or tingling), or the stomach (early feeling of fullness), enlarged spleen with abdominal discomfort. Other symptoms include fevers, chills, unexplained weight loss, night sweats, itching and lack of energy.

Non-Hodgkin's lymphomas are staged according to Ann Arbor staging system.[26] NHL is further classified with letters. An "A" or "B" designation indicates whether the person with lymphoma had symptoms or not (A- No symptoms. B – Symptoms like Fever (temperature > 38°C), drenching night sweats, and unexplained loss of more than 10% of body weight within the previous 6 months).

RESULTS:

In our study of total 127 patients, 78 (61.4%) patients were males and 49 (38.6%) were females. Among them 26 (20.5 %) patients of NHL had HBV infection and 101 (79.5%) were non-infected. Table 1

There were 18 (14.2%) patients in < 20 years of age, while 30 patients (23.6 %) in 21 – 40 years of age, 35 patients (27.6%) in 41 – 60 years of age, and 44 patients (34.6%) in > 60 years of age. Maximum infected age group was 41 – 60 years, 11 (42.3%). Table 2

B symptoms were present in 77 (60.6%) patients out of 127, and among them 17 (22.1%) were infected, and 60 (77.9%) were non-infected. Table 3.

In our study, there were 38 (29.9%) patients of low grade of

TABLE 1
HBV STATUS * GENDER DISTRIBUTION

			Gender		Total
			Male	Female	
HBV Status	Positive	Count	15	11	26
		% within HBV Status	57.7%	42.3%	100.0%
		% of Total	11.8%	8.7%	20.5%
	Negative	Count	63	38	101
		% within HBV Status	62.4%	37.6%	100.0%
		% of Total	49.6%	29.9%	79.5%
Total		Count	78	49	127
		% within HBV Status	61.4%	38.6%	100.0%
		% of Total	61.4%	38.6%	100.0%

TABLE 3
HBV STATUS * SYMPTOMS OF NHL

			Symptoms		Total
			A Symptoms	B Symptoms	
HBV Status	Positive	Count	9	17	26
		% within Symptoms	18.0%	22.1%	20.5%
		% of Total	7.1%	13.4%	20.5%
	Negative	Count	41	60	101
		% within Symptoms	82.0%	77.9%	79.5%
		% of Total	32.3%	47.2%	79.5%
Total		Count	50	77	127
		% within Symptoms	100.0%	100.0%	100.0%
		% of Total	39.4%	60.6%	100.0%

TABLE 2
HBV STATUS * AGE CATEGORY OF NHL

			Age Category				Total
			<20y	21-40	41-60	>61y	
HBV Status	Positive	Count	5	1	11	9	26
		% within HBV Status	19.2%	3.8%	42.3%	34.6%	100.0%
		% of Total	3.9%	.8%	8.7%	7.1%	20.5%
	Negative	Count	13	29	24	35	101
		% within HBV Status	12.9%	28.7%	23.8%	34.7%	100.0%
		% of Total	10.2%	22.8%	18.9%	27.6%	79.5%
Total		Count	18	30	35	44	127
		% within HBV Status	14.2%	23.6%	27.6%	34.6%	100.0%
		% of Total	14.2%	23.6%	27.6%	34.6%	100.0%

NHL, 30 (23.6%) patients of intermediate grade and in high-grade 59 (46.5%) patients. High-grade patients were mostly infected 14 (53.8%). Table 4

In our study out of 127 NHL patients, 2 (1.6 %) patients were of stage I, 43 (33.9%) of stage II, 52 (40.9 %) of stage III and 30 (23.6 %) of stage IV. Mostly infected stage patients were of stage 1V, 11 (42.3%). Table 5

DISCUSSION:

HBV infection is a major public health problem and recent studies have documented that HBV is also lymphotropic which can predispose to lymph proliferative disorders as NHL.[12] Initial evidence of this association came from high prevalence or frequency of HBV in patients of NHL as compared to general population, blood donors and controls. This association was independent of age, sex, race, transfusion history, smoking, alcohol intake and HCV infection. Later isolation of HBV DNA and HBsAg from reticuloendothelial cells, lymphocytes and endothelial lining of vascular bed supported this association.[27] It was also observed that HBV infection in NHL had prognostic significance, as HBV infected NHL patients presented at a young age, had higher stages and or grades and life expectancy was negatively infuenced.[28]

There is no local data available on prevalence or frequency of HBV in NHL, majority of international data is focusing on association of HCV with NHL, and limited research data is available on prevalence or frequency of HBV in patients of NHL. Therefore, our study is to determine the magnitude of HBV infection in patients of NHL. This study also analyzed variables as age, gender,

TABLE 4
HBV STATUS * GRADE OF NHL

			Grade of NHL			
			Low Grade	Intermediate Grade	High Grade	Total
HBV Status	Positive	Count	5	7	14	26
		% within HBV Status	19.2%	26.9%	53.8%	100.0%
		% of Total	3.9%	5.5%	11.0%	20.5%
	Negative	Count	33	23	45	101
		% within HBV Status	32.7%	22.8%	44.6%	100.0%
		% of Total	26.0%	18.1%	35.4%	79.5%
Total		Count	38	30	59	127
		% within HBV Status	29.9%	23.6%	46.5%	100.0%
		% of Total	29.9%	23.6%	46.5%	100.0%

TABLE 5
HBV STATUS * STAGE OF NHL

			Stage of NHL				
			Stage I	Stage II	Stage III	Stage IV	Total
HBV Status	Positive	Count	1	7	7	11	26
		% within HBV Status	3.8%	26.9%	26.9%	42.3%	100.0%
		% of Total	.8%	5.5%	5.5%	8.7%	20.5%
	Negative	Count	1	36	45	19	101
		% within HBV Status	1.0%	35.6%	44.6%	18.8%	100.0%
		% of Total	.8%	28.3%	35.4%	15.0%	79.5%
Total		Count	2	43	52	30	127
		% within HBV Status	1.6%	33.9%	40.9%	23.6%	100.0%
		% of Total	1.6%	33.9%	40.9%	23.6%	100.0%

stage and grade of NHL patients in HBV infected and non-infected patients.

In our study, the frequency was much higher (20.5%) than the frequency of HBV in general population in Pakistan.[3] This favors the observation of Chen MH et al.[13] They found that 23.5% NHL patients were having HBV infection.

In our study, frequency of HBV in patients of NHL was independent of age and gender.

A South Korean study [14] shows HBsAg positive in 12.6% patients as compared to controls of 4.7%. There was no difference in stage or grade of HBV infected NHL patients but HBV infected patients presented at a young age.

A six year cohort study conducted in USA[15], HBV infection was 2.8 time more frequent. This study supports our results that, HBV infection was 2 – 3 fold more frequent in NHL when compared to our general population.

A Chinese study [16] conducted in 2007, reported prevalence of HBV in NHL at 30.2%. It was concluded from this study that HBV infection is higher in patients of NHL and NHL developed 9.5 years earlier in HBV infected patients and was associated with B - cell NHL rather than T – cell NHL. Our study differ from this study on earlier age of onset, as our study showed that frequency of HBV in NHL was independent of age.

Park et al.[17] observed prevalence of HBV 15.1% as compared to 8.1% in controls. The points of difference were that our study did not enroll a control group and did not assess the role of HCV, as it was not the aim of our study. Mehdi SR et al.[18] observed prevalence of 11.2% for HBV, their high frequency of HBV in NHL was as of our study, though in absolute term it was less than what we reported. The point of difference was that our study was a cross sectional study and their's was case control, second they enrolled male patients and assessed the impact of HCV in NHL. Marcucci et al.[19] observed prevalence of HBV 8.5% as compared to 1.5% of Italian general population and control group of 2.8%. Most of the studies discussed above, reported high HBV infection rate in NHL patients. Only the results of Chinese study, Chen MH et al, and Wang et al.[20] reported frequencies higher than observed in our study. One possible explanation for this could be high prevalence of HBV in rural areas of Pakistan, where our study was conducted.

Our study also observed higher stage and grades of NHL in HBV infected patients. 42.3% of stage 1V HBV infected patients had

high grade NHL as compared to 18.8% in non-infected patients. This favors the study of Wang et al.[20] where HBV infected patients had advance stage of NHL (57%) and younger age of onset.

The above discussion based on the international literature has showed that Hepatitis B and Non Hodgkin Lymphoma have some pre despondence or association. Despite that, these two monsters are common in our region. The current trend of both these disorders is on remarkable increase. Therefore, we would propose a large-scale epidemiological study, not only to gather the relevant data but also focusing on some of the factors and reasons for co existence of HBV and Non Hodgkin Lymphoma.

CONCLUSION:

This study concluded that, high percentage of Non Hodgkin Lymphoma patients have hepatitis B infection. Out of 127 Non Hodgkin Lymphoma patients, 26 (20.5%) patients were positive for HBV. This was independent of age and gender. Beside, these patients of infected NHL had higher grade and stage.

REFERENCES:

1. Lowrence S, Friedman MD. Liver,biliary tract, & pancreatic disorder. 2010 current medical diagnosis & treatment, forty-ninth edition: 608-12
2. World Health Organization (who.int) Hepatitis B Key Facts.Fact sheet No. 204 Revised August 2008.[updated 10 April 2010]Available from http:// www.who.int/mediacentre/factsheets/ fs204/en/
3. Pakistan Medical Research Council Islamabad. National Survey (Jul 2007 - Jun 2008) on Prevalence of Hepatitis B & C in General Population of Pakistan.[cited 2010 apr 8]. Available from http/ www.pmrc.org.pk/ hepatitisbc.htm.
4. Mujeeb SA, Hussain W, Haq A. Prevalence of hepatitis B infection in professional and family donors. J Pak Med Assoc 1994; 44: 226.
5. Tanveer A, Batool K, Qureshi AW. Prevalence of hepatitis B and C at University of the Punjab, Quaid-e-Azam Campus Lahore. ARPN Agricultural Biolog Sci 2008 Mar; 3(2): 30-2.
6. Dienstag JL. Chronic Hepatitis. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jamesons JL, et al. Harrison's principles of internal medicine, 17th ed: USA: McGraw Hill, 2008: 1955-69.
7. Koff RS. Immunologically mediated extrahepatic manifestations of viral hepatitis. In: Krawitt EL, Wiesner RH eds. Autoimmune liver diseases. New York: Raven press, 1991: 233-45.
8. Wrzolkowa T, Zurowska A, Mrozowicz-Picken M. Hepatitis B virus associated glomerulonephritis: electron microscopic studies in 98 children. Am J Kidney Dis 1991; 18: 306-12.
9. Lohr H, Goergen B, Weber W, Godderz W, Meyer zum Buschenfelde KH, Gerken G et al.. Mixed cryoglobulinemia type II in chronic hepatitis B associated with HBe-minus HBV mutant: Cellular immune reaction and response to interferon treatment. J Med Virol 1994; 44: 330-5.
10. Rich AR. The role of hypersensitivity in periarteritis nodosa as indicated by seven cases developing during serum sickness and sulfonamide therapy. Bull Johns Hopkins 1942; 71 :123-40.
11. Lao TT, Tse KY, Chan LY. HBsAg carrier status and the association between gestational diabetes with increased serum ferritin concentration in Chinese women. Diabetic care 2003; 26: 3011-6.
12. Kuniyoshi M, Nakamuta M, Sakai H, Enjoji M, Kinukawa N, Kotoh K, *et al.* Prevalence of hepatitis B or C virus infections in patients with non-Hodgkin's lymphoma. J Gastroenterol Hepatol 2001 Feb; 16(2): 215-9.
13. Chen MH, Hsiao LT, Chiou TJ, Liu JH, Gau JP, Teng HW, et al. High prevalence of occult hepatitis B virus infection in patients with B cell non-Hodgkin's lymphoma. Ann Haematol 2008 Jun; 87(6): 475-80.
14. Kim JH, Bang YJ, Park BJ, Yoo T, Kim CW, Kim TY, et al. Hepatitis B virus infection and B-cell non-Hodgkin's lymphoma in a hepatitis B endemic area: a case-control study. Jpn J Cancer Res 2002; 93: 471-7.
15. Ulcickas Yood M, Quesenberry CP Jr, Guo D, Caldwell C, Wells K, Shan J, et al. Incidence of non-Hodgkin's lymphoma among individuals with chronic hepatitis B virus infection. Hepatology 2007 Jul; 46(1): 107-12.
16. Wang F, Xu RH, Han B, Shi Y, Luo HY, Jiang WQ, et al. High Incidence of Hepatitis B Virus Infection in B-cell Subtype Non-Hodgkin Lymphoma Compared With Other Cancers. Cancer 2007 Apr; 109(7): 1360-4.
17. Park SC, Jeong SH, Kim J, Han CJ, Kim YC, Choi KS, et al. High prevalence of hepatitis B virus infection in patients with B-cell non-Hodgkin's lymphoma in Korea. J Med Virol 2008 Jun; 80(6): 960-6.
18. Mehdi SR, Ajlan ARA. Hepatitis B & C virus infection in cases of non Hodgkin's lymphoma in Saudi Arabia. Turk J Hematol 2006; 23: 200-4.
19. Marcucci F, Mele A, Spada E, Candido A, Bianco E, Pulsoni A, et al. High prevalence of hepatitis B virus infection in B-cell non-Hodgkin's lymphoma . Heamotogica 2006 Apr; 91(4): 554-7.
20. Wang F, Xu RH, Luo HY, Zhang DS, Jiang WQ, Huang HQ. Clinical and prognostic analysis of hepatitis B virus infection in diffuse large B-cell lymphoma. BMC Cancer 2008 Apr; 8: 115-8.
21. Johnston, J. Martin. "Non-Hodgkin Lymphoma." eMedicine.com. Dec. 5, 2008. <http://emedicine.medscape.com/article/987540-overview>.
22. United States. National Cancer Institute. U.S. National Institutes of Health. "Non-Hodgkin Lymphoma." Feb. 12, 2008. <http://www.cancer.gov/cancertopics/ wyntk/non-hodgkin-lymphoma>.
23. Cartwright R, Brincker H, Carli PM, Clayden D, Coebergh JW, Jack A et al. The rise in incidence of lymphomas in Europe 1985-1992. European Journal of Cancer 1999; 35(4): 627-33.
24. Harris NL, Jaffe ES, Diebold J, Flandrin G, Mullerhermelink HK, Vardiman J. Lymphoma classification— from controversy to consensus: the R.E.A.L. and WHO classification of lymphoid neoplasms. Ann Oncol. 2000; 11 Suppl 1:3–10.
25. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2001
26. Diehl V, Thomas RK, Re D et al. Hodgkin's lymphoma, diagnosis and treatment. Lancet Oncology 2004; 5: 19–26
27. Pontisso P, Poon MC, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in mononuclear blood cells. Br Med J 1984; 288: 1563–6.
28. Liang RH, Lok AS, Lai CL, Chan TK, Todd D, Chiu EK. Hepatitis B infection in patients with lymphomas. Hematol Oncol 1990; 8(5): 261-70.