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RESEARCH PAPER

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Immunohistochemical Detection of Epstein-Barr virus (LMP-1) among Non Hodgkin Lymphomas Patients in Sudan

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ABSTRACT

The presence of Epstein-Barr virus (EBV) in Non-Hodgkin's lymphoma can be identified by immunohistochemistry for detection of EBV latent membrane protein (LMP). The role of EBV as an etiologic agent in the development of non-Hodgkin lymphoma has been supported by detection of high levels of latent membrane protein 1 (LMP-1) expression in tumors. The objective of our study was to determine a value for non-Hodgkin lymphoma patients using EBV LMP-1 immunostaining in Sudan. This study was carried at the Radio Isotope Center Khartoum (RICK)-Khartoum state –Sudan from January 2014 to July 2015. It was a cross sectional study.

A total of 59 patients who were diagnosed with various subtypes of NHL after histological and EBV LMP-1 immunohistochemical evaluation were studied. Sampling technique was non-probability purposive. Statistical analysis was achieved using SPSS version 11.5. Mean and SD were calculated for quantitative variables like patient age. Frequencies and percentages were calculated for qualitative variables like subgroup of NHL, results outcome of IHC for EBV and gender distribution, for quantitative data Chi - test and correlation were used for qualitative data (significance level were set at P≤ 0.05). A total of 41 (69.4%) were male and 18 (30.6%) were female. LMP-1 immunohistochemical marker was positive in (30.5%) (18/59) cases in all Non Hodgkin`s lymphoma. The expression of LMP-1 among NHL subtypes as follow; (36.8%) (7/19) of diffuse large B cell lymphoma, (80%) (8/10) cases of Burkitt lymphoma, one out of two (50%) positive cases of follicular lymphoma, (7.1%) (1/14) cases of small lymphocytic lymphoma and no expression of EBV LMP-1 antibody were reported in mantle cell lymphoma. In our study, frequency of EBV in NHL is 30.5% and is mostly seen in Burkitt lymphoma and diffuse large B cell lymphoma. This requires further evaluation to find out whether this positivity is due to co-infection or has a role in pathogenesis.

Keywords: Epstein, Barr virus, NHL, Immunohistochemistry and Latent membrane protein 1.

INTRODUCTION

Non-Hodgkin's Lymphoma (NHL) is a cancer of the lymphatic system (Evans and Hancock.2003; National Cancer Institute. 2005)**.** Malignant lymphoma is a primary malignant neoplasm of lymphoid tissue arising from the expansion of malignantly transformed lymphocytes, which may contain one or more genetic abnormalities (JCE. Underwood and Joseph Hunter. 2004). It is divided into two broad categories; Hodgkin's lymphoma and Non-Hodgkin's lymphomas (NHLs). Genetic alternations, viruses and environmental agents as well as radiotherapy and chemotherapy are implicated as etiologic factors (Robbins and Cotran. 2005). Non-Hodgkin´s lymphomas (NHLs) constitute heterogeneous group of malignant lymphoproliferative disorders. It can arise from nodal or extra nodal locations and spread in unpredictable fashion. Two thirds of NHLs and virtually all cases of Hodgkin´s lymphomas present with non-tender nodal enlargement (often greater than 2 cm). The lymphadenopathy can be localized or generalized. The remaining one third of NHLs arises at extra nodal sites such as skin, stomach and brain. The extra nodal location found in approximately 20% of patients with limited stage high grade disease (Sir Robert, et al .1998)

Epstein Barr virus (EBV) is an important example of a transforming virus implicated in several NHL subtypes (Beltran et al., 2011). EBV is usually acquired in early childhood in developing countries. In developed countries, primary infection in adolescence is associated with the clinical syndrome of infectious mononucleosis. Following primary infection, EBV persists lifelong in the host in a latent state in memory B lymphocytes. EBV readily transforms B lymphocytes in vitro. Multiple EBV proteins can be expressed in infected lymphocytes, among which latent membrane protein-1 is thought to be most important for transformation. In healthy infected individuals, outgrowth of EBV-transformed B lymphocytes is prevented by the presence of intact T lymphocyte-mediated immunity (Nourse et al., 2012).

The expression of Epstein-Barr virus in Non-Hodgkin's lymphoma can be identified by immunohistochemistry for detection of Epstein-Barr virus latent membrane protein (LMP). The role of Epstein-Barr virus as etiologic agent in the development of Non-Hodgkin lymphoma has been supported by detection of high levels of latent membrane protein 1 (LMP-1) expression in these tumors. The prevalence of EBV in NHL is 10% (Matsushita et al., 2012).

MATERIALS AND METHODS

This study was carried out at the Radio Isotope Center Khartoum (RICK)-Khartoum state –Sudan from January 2014 to July 2015. It was a cross sectional study.. Sampling technique was non-probability purposive. The study group comprised Formalin –fixed paraffin embedded Lymph node biopsies of fifty nine (59) comprised non malignant lymphomas, all cases were selected from the surgical pathology obtained from the archives of Radiation Isotope Center Khartoum (*RICK*) after taking the numbers of blocks from the patient records data of the following years (2011, 2012, 2013 and 2014) all the histological samples were confirmed, by histopathologist. Further sub typing of NHL was done according to WHO classification of lymphomas by using a panel of immunohistochemical markers. EBV was labeled as positive on finding cytoplasmic staining of tumor cells by EBV-LMP-1 antibody according to Standard Strepto Avididin Biotin (Thermo Fisher) protocol

The Immunohistochemical procedure was done as follows

Three microns (4µm) sections from formalin-fixed, paraffin-embedded were cut and mounted onto salinized slides (Fisher brand). Monoclonal antibody (LMP- 1) was used as manufacture instructions to detect presence of (*EBV*) .All sections were deparaffinized in two changes of xylene for 10 minutes in each change, then rehydrated in descending changes of ethanol as follows; sections were placed in two changes of absolute ethanol for 5 minutes in each change and then were placed in 90% ethanol for 3 minutes, and then were placed in 70% ethanol for 2 minutes, and then were washed in distilled water for 2 minutes. Sections were steamed for antigen retrieval for (*EBV*) using (PT link) in 10mM-citrate buffer Hydrochloric acid (*HCl*) (pH 7.6) for 20 minutes. Then slides were cooled in a sink containing cold tap water, the slides were removed once the buffer at room temperature, slides were rinsed in running tap water and placed in TBS at pH 7.4. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min, then ultra V block was applied and incubated for 8 min and then Slides were incubated with 100 µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then were rinsed in Phosphate buffer saline. The primary antibody for EBV (Monoclonal antibody (LMP-1) was ready to use (Thermo Fisher). After washing with PBS for 3 minutes, binding of antibodies was detected by incubating with biotinylated goat anti polyvalent for 15 minutes, followed by incubating for 15 min with streptavidin peroxidase (Thermo Fisher kit). the slides then were be washed with two rinses of TBS pH 7.4 for 1-2 minutes followed, then the slides were be covered by 0.01% H_2O_2 and 0.05 3.3 diamino benzidine tetra hydro chloride (DAB) chromogen in Tris HCl pH 7.6 for 5 minutes at room temperature, (Thermo Fisher) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex. the sections was counter stained with Mayer's Haematoxylin for one minute, washed in tap water for 2

minutes, dehydrated through 3 different baths of absolute alcohol, placed in xylene, mounted in (*DPX*)**.** For each run of staining, positive and negative control slides was also prepared. The positive control slides contain the antigen under investigation and the negative control slides prepared from the same tissue block, but were incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

RESULTS

Total of (59) cases of non Hodgkin lymphomas were included in the study, of which 41 (69.4%) patients were males and 18 (30.6%) were females as illustrated in (table-1). Considering the frequency of subtypes of Non Hodgkin`s Lymphomas, (44%) (26/59) were for Diffuse large B cell lymphoma, (17%) (10/59) for Burkitt lymphoma, (25.4%) (15/59) for Follicular lymphoma, (3.3%) (2/59), (7%) (4/59) for T cell lymphoma and (3.3%) (2/59) for Marginal zone lymphoma as in showed in (table-2). As the age categorized into three groups, (less than 30 yrs) represent the children and young adults), (30-50 yrs) represent the middle age and (above 50 yrs) represent the elderly , the frequency of histological types of Non Hodgkin`s lymphomas were affect by age as the (P. value= 0.000). Burkitt lymphoma and diffuse large B cell lymphoma affected the children and young adults, while Small lymphocytic lymphoma affected the elderly, as in (table-4). LMP-1 immunohistochemical marker was positive in 18 (30.5%) cases and negative in 41 (69.5%) cases as demonstrated in (table-3). (Table-5) shows expression of EBV LMP1 antibody in different types of Non Hodgkin lymphomas. 7 out of 19 (36.8%) cases of diffuse large B cell lymphoma, 8 out of 10 (80%) cases of Burkitt lymphoma, one out of two (50%) positive cases of follicular lymphoma cases, one out of 14 (7.1%) cases of small lymphocytic lymphoma and no expression of EBV LMP-1 antibody were reported in mantle cell lymphoma.

Table 1. Distribution of study population according to the gender.

Gender	Frequency	Present
Male	41	69.4
Female	18	30.6
Total	59	100

Table 2. The frequency of non Hodgkin`s lymphoma subtypes.

NH L subtype	Frequency	Present
D L B cell lymphoma	26	44.1
Burkitt lymphoma	10	16.9
Small .L lymphoma	15	25.4
Follicular lymphoma	2	3.4
T cell NHL	4	6.8
Marginal zone lymphoma	4	3.4
Total	59	100

Table 3. The immunostain positive rate among study population.

I H C result	Frequency	Present
Positive	18	30.5
Negative	41	69.5
Total	59	100

Table 4. The association of age and Different types of non Hodgkin`s lymphomas.

Type of NHL	Age group			Total	P. value
	Less than 30yrs	30 to 50 yrs	More than 50yrs		
D L B cell lymphoma	14	6	6	26	0.000
Burkitt lymphoma	9	1	0	10	
Small .L lymphoma	1	3	11	15	
Follicular lymphoma	0	2	0	2	
T cell NHL	1	2	1	4	
Marginal zone lymphoma	0	0	2	2	
Total	25	14	20	59	

Table 5. The frequency of immunostain result among different type of NHL.

Type of NHL	**Immunostain result**		**Total**	**P. value**
	Positive	**Negative**		
D L B cell lymphoma	**7**	**19**	**26**	**0.04**
Burkitt lymphoma	**8**	**2**	**10**	
Small .L lymphoma	**1**	**14**	**15**	
Follicular lymphoma	**1**	**1**	**2**	
T cell NHL	**1**	**3**	**4**	
Marginal zone lymphoma	**0**	**2**	**2**	
Total	**18**	**41**	**59**	

DISCUSSION

Herpes viruses, EBV is an enveloped virus that contains a DNA core surrounded by an icosahedral nucleocapsid and a tegument. Family members include herpes simplex I and II and varicella-zoster virus (alpha virus subfamily), cytomegalovirus and human herpes virus 6 and 7 (beta herpesvirus subfamily), and human herpes virus 8 and EBV (gamma herpes virus subfamily) (Mushtaq et al., 2008; Nourse et al., 2012).

Despite our growing understanding of the role of EBV in the pathogenesis of disease, the optimal management of EBV associated tumors remains unsatisfactory. Exploration of antiviral agents, immune-based therapies, and specific monoclonal antibodies is, however, proceeding with encouraging results (Chabay et al., 2009; Nourse et al., 2012).

The present study reveals that types of Non Hodgkin`s lymphomas were affect by age as the (P. value= 0.000). Burkitt

lymphoma and diffuse large B cell lymphoma affected the children and young adults, while Small lymphocytic lymphoma affected the elderly, comparatively similar results of the study in Egypt (Karen et al., 2004) showing 49.5% of NHL cases below 50 years.

Globally, NHL is more common in males as compared to females. Similar trend was seen in this study in which (69.4%) of patients were males and (30.6%) were females. These results were similar to the findings of (Mushtaq, *et al*. 2008; Jamal *et al*. 2006), "in which males comprised 68% of the patients". In addition to that, the present study represent that , a diffuse Large B-cell lymphoma was the most common type of NHL (44%), followed by small lymphocytic lymphoma (25.4%), Burkitt lymphoma (17%), follicular lymphoma (3.3%), T-lymphoblastic lymphoma (7%), marginal zone lymphoma (3.3%). The same result was obtained in Pakistan by (Ishtiag, *et al*. 2013) "who concluded that, (the most common subtypes of non Hodgkin lymphoma is diffuse large B cell lymphoma". The explanation for variations in the prevalence of NHL in various studies might be due to different study methodologies of classifications, difference in sample size, and it also may be due to some environmental factors

Overall, EBV LMP-1detected in our study among all NHL was (30.5%) (18 out of 59 cases) , the high frequency was reported in Burkitt lymphoma (80%) followed by diffuse large B cell lymphoma (36.8%). The study clarified that Burkitt's is most commonly NHL associated with EBV infection, this may be due to the association with Plasmodium Falciparum in Africa. . Numerous frequency of EBV positivity in NHL case were recorded in different studies, Gonin et al. showed the positivity of EBV in 30% of NHL) Gonin, et al. 20011). Others illustrated the EBV positivity was seen in 12.7% of (9 / 71) cases while the high EBV genomes were detected in 68% of all NHL. (Cahir et al, 2002).Furthermore, Tumwine et al showed the frequency of expression of LMP-1 of EBV was detected in NHL patients (34.7%) (Tumwine et al .2010). When compared with previous results mentioned above, we can see many of them in agreement with our results, but it seem to be near the last study which conducted in Uganda, may be due to the same economic and the environmental factors, that present in the geographical neighbor country

CONCLUSION

Frequency of EBV in NHL in our study is 30.5% and it is mostly seen in Burkitt lymphoma (80%) followed by diffuse large B cell lymphoma (36.8%). This requires further evaluation to find out whether this positivity is due to co-infection or has a role in pathogenesis.

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REFERENCES

Beltran, B.E., Morales, D., Quiñones, P., et al (2011). EBV-positive diffuse large b-cell lymphoma in young immunocompetent individuals. *Clin Lymphoma Myeloma Leuk*, **11**, 512-6.

Cahir Mc Farland, E., and Kieff, E. (2002). NF-kB inhibition in EBV-transformed lymphoblastoid cell lines. *Recent Results Cancer Res*; 159: 44-8.

Chabay, P., De Matteo, E., Lorenzetti, M., et al (2009). Low 3966 DOI: http://dx.doi.org/10.7314/APJCP.2013.14.6.3963. Epstein-Barr Virus Expression in Non-Hodgkin Lymphomas, *Asian Pacific*

Journal of Cancer Prevention, Vol. 14, 2013, frequency of Epstein Barr virus association and high frequency of p 53 over expression in an Argentinean pediatric T-cell lymphoma series. *Pediatr Dev Pathol*, 12, 28-34.

Evans, L.S. and Hancock, B.W. (2003). Non-Hodgkin lymphoma. Lancet, 362: 139-146. Pub Med Abstract | Publisher Full Text.

Gonin, J., Larousserie, F., Bastard, C., et al. (2011). Epstein-Barr virus-induced gene 3 (EBI3): a novel diagnosis marker in Burkitt lymphoma and diffuse large B-cell lymphoma. P LoS One, 6, 24617.

Ishtiaq, S., Hassan, U., Mushtaq, S. and Akhtar, N., 2013. Determination of frequency of epstein-barr virus in non-Hodgkin lymphomas Using EBV latent membrane protein 1 (EBV-LMP1) immunohistochemical staining. *Asian Pac J Cancer Prev.;* 14(**6**)**:** 3963-7.

Jamal, S., Moghal, S., Mamoon, N., Mushtaq, S., Luqman, M. and Anwar, M. (2006). The pattern of malignant tumours: tumour registry data analysis, AFIP, Rawalpindi, Pakistan (1992-2001). *J Pak Med Assoc*; 56(**8**)**:** 359-62.

JCE. Underwood, Joseph Hunter, (2004). General and systemic pathology. London. Churchill Livingstone .

Karen, D.C., Christopher, A.L., Soeir, Abdel-Latif, E., et al (2004). Case-control study of non-Hodgkin's lymphoma and hepatitis C virus infection in Egypt. *Int J Epidemiol*, **33**, 1034-39.

Matsushita, H., Nakamura, N., Tanaka, Y., et al (2012). Clinical and pathological features of B-cell non-Hodgkin lymphomas lacking the surface expression of immunoglobulin light chains. *Clin Chem Lab Med*, **50**, 1665-70.

Mushtaq, S., Akhtar, N., Jamal, S., Mamoon, N., Khadim, T., Sarfaraz, T. and Waqar, A. (2008). Malignant Lymphomas in Pakistan According to WHO Classification of Lymphoid Neoplasms. *Asian Pac J Cancer Prev*.; 9 (**2**)**:**229-32.

National Cancer Institute, (2005). What you need to know about Non-Hodgkin's Lymphoma". In NIH Publication; No. 05-1567. Bethesda, Maryland.

Nourse, J.P., Crooks, P., Keane, C., et al (2012). Expression profiling of Epstein-Barr virus-encoded microRNAs from paraffin-embedded formalin-fixed primary Epstein-Barr virus-positive B-cell lymphoma samples. *J Virol Methods*, **184**, 46-54.

Robbins and Cotran, (2005). Pathologic basis of disease. USA. Philidelphia. Elsevier Saunders press.

Sir Robert Muir, Roderik, N. M. Mac Sween and Keith Whaley (1998). Text book of pathology. 1998. uk: London. Hodder Arnold Publication.

Tumwine, L.K., Orem, J., Kerchan, P., et al (2010). EBV, HHV 8 and HIV in B cell non Hodgkin lymphoma in Kampala, Uganda. Infect Agent Cancer, 5, 12.

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