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Immunohistochemical Detection of Epstein Barr Viruse among Lymphoma Sudanese Patients

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Abstract*: This is retrospective descriptive case study aimed to detect the presence of Epstein Barr virus in lymphoma patient's samples using immunohistochemical method. Sixty paraffin embedded blocks previously diagnosed as lymph node lesions were collected. Samples include 50 malignant tumors (11 Hodgkin lymphoma and 39 non Hodgkin lymphoma) and 10 samples were benign tumors. One section of 3µm thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (modified new indirect method) for detection of EBV. Data was collected from patient's files and the obtained results were analyzed using SPSS computer program. The patient's age ranged between 7month and 80 years with mean 40 years, most patients 33 (55%) were more than 40 years and the remaining 27 were less than 40 years representing (45%) patients were more than 40 years. The majority of patients were males and the male: female ratio was 2.3:1 representing 42(70%) males and the remaining 18(30%) were females. Immunohistochemical detection of EBV was revealed positive result in 3/50 samples and negative result in 47/50 in malignant samples while all benign tumors showed negative result for EBV, with insignificant statistical association between EBV expression and histopathology diagnosis (P=0.427). The study concludes that EBV is detected in few samples of lymphoma samples.*

Keywords— Epstein Barr Viruse; Lymphoma; Immunohistochemistry.

1. INTRODUCTION

Lymphoma is the cancer of the lymph system (or lymphatic system). It is characterized by the formation of solid tumors in the immune system [(1)].

Lymphoma is the tenth most common cancer worldwide, with around 452.000 new cases diagnosed in 2012 with percentage 3.2 % of total cases of the diagnosed cancers. In Sudan lymphoma is third type of cancers in terms of occurrence with (rate = 8.2 per 100,000) [(2)].

Based on the world health organization (WHO) classification lymphoma was classified into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) [(3)]. The WHO classified lymphoid neoplasm to main categories which comprised mature B-cell neoplasms, mature T-cell and NK-cell neoplasms, Hodgkin lymphoma, post transplantation lymph proliferative disorders (PTLDs) [(4)].

Epstein-Barr virus (EBV), a human lymphotropic herpes virus, it is associated with a number of malignancies including Hodgkin's disease, B cell lymphomas, and nasopharyngeal carcinoma [(5)].

Also causes infectious mononucleosis and Burkitt lymphoma (BL) [(6)]. EBV is a ubiquitous virus that infects at least 95% of the population. Most persons are infected during infancy and early childhood and are asymptomatic or have nonspecific symptoms [(7)]. Infection of adolescents and young adults with EBV often result with fever, lymphadenopathy, sore throat, and splenomegaly, fatigue and myalgias [(8)]. Males with the X-linked lymph proliferative disease often develop fatal infectious mononucleosis during primary EBV infection [(9)]. Chronic active EBV disease (CAEBV) is a lymphoproliferative disorder characterized by markedly elevated levels of antibody to EBV or EBV DNA in the blood and EBV RNA or protein in lymphocytes in tissues [(10)].

2. Materials and methods:

2.1 Materials:

Archived tissue blocks obtained from Sudanese lymph nodes samples previously diagnosed as lymphoma and hyperplasia were selected for this study.

2.2 Study design

This is a descriptive retrospective case study aimed to detect the presence of EBV in lymphoma patient's samples using immunohistochemistry.

3. Methods:

3.1 Sample processing:

Section to be stained were cut at 3µm thickness by rotary microtome, mounted in positively charged glass slides and put at 60˚C oven for 30 minutes.

3.2 Immunohistochemical stain

One section of 3µm thickness was obtained from each paraffin block using a SLEE CUT 5062 rotary microtome, then was placed on a positively charged slide and dried overnight at 58° and immunostained using monoclonal primary antibody by biotinylated secondary antibody indirect technique as follows:

Sections were loaded into Ventana Bench Mark GX autostaine, they were deparaffinized in EZ prep solution, and then they covered with cell conditioning 1 (CC1) to unmask the antigenicity by selecting mild CC1, standard CC1 for sixty minutes. The activity of endogenous peroxidase was

blocked by the Inhibitor (3% hydrogen peroxide (H2O2)), and the endogenous biotin was blocked by biotin blocking solution. Then the sections were treated with the primary antibody (Anti-Epstein-Barr virus (LMP- 1)) for sixteen minutes, and then slides were incubated in secondary antibody (biotinylated antibody). Then the slides were incubated in Horse Radish Peroxidase (HRP) of concentration less than 300µg/mL in the presence of Copper (5g/L CuSO4) as a co-factor, and then diaminobenzidene (DAB) substrate (2g/L) was added to the sections to visualize the reaction producing dark brown color. Between each two steps slides were washed with the reaction buffer. The sections counterstained with Hematoxylin II (60%) and finally were treated with bluing reagent (0.1 M Li2CO3, 0.5 M Na2CO3). Slides were moved from instrument, dehydrated in alcohol, cleared in xylene and mounted with DPX [(11)].

3.3 Result interpretation:

All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining. Detection of more than five cells with brown cytoplasm per one field considered as positive result.

3.4 Data analysis:

Data analysis was done using SPSS 20 computer program. Frequencies mean and Chi –square test values were calculated.

3.5 Ethical consideration:

Samples were collected after taking ethical acceptance from hospital administration.

4. Results

A total of 50 samples of patients with lymph node disorders were investigated, 40 of them were lymphoma representing 83.3%, and the remaining 10 (16.7%) were benign (Table 1). The age of study population ranged between 7 month and 80 years with mean of age 40 years. Patients less than 40 years were 27 (45%) and older than 40 years were 33 (55%) (Table 2).

The sex of study population revealed that 42(70%) patients were males and 18 (30%) patients were females (Table 3).

EBV revealed positive expression in 3(5%) of lymphoma samples and negative expression in 47(78.3%) samples ,while all hyperplasia samples showed negative expression of EBV (Table 4).

Table (1): Histopathology diagnosis among the study samples

Sample	Histopathology diagnosis	Frequency	Percent
Malignant	Hodghkin lymphoma	11	18.3%
	Non-Hodghkin lymphoma	39	65%
Benign	Hyperplasia	10	16.7%
Total		60	100%

Table (2): Distribution of age groups among the study population

Age (years)	Frequency	Percent
40 years and less	27	45%
More than 40	33	55%
Total	60	100%

Table (3): The distribution of sex among study population

Sex	Frequency	Percent
Male	42	70%
Female	18	30%
Total	60	100%

Table (4): Relation between the expression of EBV and histopathological diagnosis.

Histopathology diagnosis	EBV expression		Total	P.value
	Positive	Negative		
	N (%)	N (%)	N (%)	
Malignant	3(5)	47(78.3)	50 (83.3)	0.427
Benign	0 (0)	10(16.7)	10 (16.7)	
Total	3 (5)	57 (95)	60 (100)	

5. DISCUSSION

The top most common cancers in both sexes are breast, non-Hodgkin lymphoma, leukemia, esophagus [(12)].

In this study out of sixty samples of patients with lymphoproliferative disorder were investigated by immunohistochemical method, 50 of them were lymphoma representing 83.3%, and the remaining 10(16.7%) were benign.

The age of the study population ranged between 7 month to 80 years with mean of age 40 years. Patients less than 40 years were 27(45%) and older than 40 years were 33 (55%). This mean that lymphproliferative disorder occurs in older age.This result incompatible with Abuelhassan, *et al.* [(13)], who reported that lymphoma more marked in children.

Regarding sex that males are more affected with lymphproliferative disorder than female representing (70%) and (30%) respectively. This result supported by Decaudin, *et al.* [(14)], who reported that mantle cell lymphomas are characterized by a male predominance these observations suggest a possible relation between the chromosome X and mantle cell lymphomas which has to be explored. Also supported by Grundy, *et al.* [(15)], who reported that non-Hodgkin's lymphoma was 2½ times more common among males than females. Also Yakubu, *et al.* [(16)], reported lymphoma is common in male. Also Abuelhassan, *et al.* [(13)], reported males were commonly affected.The result also supported by Abuidris, *et al.* [(17)], who reported male: female ratio of 1.6:1.

Lymphoma revealed positive expression of EBV in 3(5%) patients, while all benign lymphnode showed negative expression of EBV in10 (16.7%) patients, this result show insignificant statistical association (P value 0.427).This result supported by Salah, *et al.* [(18)], who concluded that there are no association between EBV and malignant lymphoma and therefore, cannot be used as significant

prognostic factor. Also the result supported by Ishtiaq, *et al*. [19], who reported NHL cases were 38 and only one was positive for LMP 1 (3%). Also the result compatible with Mohammad, *et al*. [20], who reported that nodal and extra nodal lymphoma are negative for EBV in IHC method. This study incompatible with Mori and Katano, [21], who reported that several subtypes of human malignant lymphomas are known to be highly associated with the EBV. All hyperplasia specimens are EBV negative compatible with Huh, *et al*. [22], who examined hyperplasia samples for the presence of the genome of Epstein-Barr virus, he concluded that there is no evidence that EBV plays any role in the pathogenesis of lymphadenitis. Also the result compatible with Jing, *et al*. [23], who reported that all hyperplasia specimens were negative for EBV. This result also incompatible with Stefan, *et al*. [24], who reported that EBV-driven B-cell lymphoproliferative disorders (LPDs) occurs in immunosuppressed patients with primary immune deficiency, or post transplantation immunosuppression or who have received other treatments. McGuire, *et al*. [25], also reported that benign lymphoepithelial lesions were positive for EBV genome.

6. CONCLUSTION

The study we concluded that EBV is detected in few samples of lymphoma samples.

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