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Epstein barr virus associated Hodgkin and non-Hodgkin lymphoma

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***Abstract*---**Background: The Epstein-Barr virus (EBV) has been linked to a variety of B-cell lymphomas, including Burkitt lymphoma (BL), classical Hodgkin lymphoma (cHL), and diffuse large B-cell lymphoma (DLBCL), at rates ranging from 5 to 10% of DLBCL cases to >95% in endemic BL. Methods: The Present study conducted in AL-Najaf Holy City; it is a cross sectional study. All patients were recruited from Middle Euphrates Cancer Research Unit Laboratory, from pre-existing samples. Tissues collected from the previously mentioned laboratory were fixed in 10% formalin and placed in a cassette before being embedded in paraffine. The tissues were examined and diagnosed as with Hodgkin disease. Eighty samples were subjected in the present study. All study group was diagnosed formerly as paraffin imbedding blocks. Molecular analysis also used to detection of EBV-LMP-1 Gene. Histopathological examination using hematoxylin and eosine stain (H&E) then examining under light microscope to check for EBV-LMP-1 protein in twenty block samples of tissues with lymphoma. Results: The result of the current study revealed that EBV-LMP-1 marker found in 12.5% of cases that had been diagnosed by PCR and IHC.

***Keywords*---**EBV, FFPET, IHC, HL, Hodgkin disease.

Introduction

Epstein Bar Virus (EBV) is a member of the herpesviridae family of viruses, subfamily gammaherpesviridae, and the genus lymphocryptovirus was later identified (Epstein & Barr, 1964;Burkitt ,1962 and Levine ,1991). It is characterized by lymphocyte tropism and the ability to live in the infected host for an extended period of time. EBV has a role in its pathogenesis of Hodgkin lymphoma, the virus is responsible for almost half of all instances of Hodgkin

lymphoma (Grewal et al. 2018). EBV infection causes immunosuppression and chronic antigenic activation, both of which are essential components of the neoplastic process(Castillo et al. 2018). Non-Hodgins's lymphoma (NHL) is considered as the most common hematological disease refers to a broad category of B-cell and T-cell proliferations. Which is a heterogeneous disease, with approximately 3% of cancer diagnoses and fatalities (de Martel et al. 2020). EBV has been linked to the pathogenesis of several types of NHL with incidences range of 0.1 to 63 % (Ding et al. 2021). Geographic location, age, sex, genetic background and socioeconomic status all affect EBV-associated lymphomas. The vast majority of the world's population is infected by the time they reach adulthood, that affects more than 90% of the world's population(Sausen et al. 2021; Hadi and Aljanaby, 2022). In children, the prevalence of EBV infection ranges from 10% to 90%, depending on their age, geographic location, and ethnicity**.** The prevalence of EBV infection among Iraqi people accounts about 79.8% (Redha et al. 2017; Aljanaby et al., 2022). EBV antibody prevalence among entering young adults at the University of Minnesota fell from 64% in 2006 to 52% in 2012. Data from the National Health and Nutrition Examination Surveys (NHANES) support this. According to NHANES, EBV antibody prevalence among US children aged 6–19 years fell from an average of 72 % in 2003–2004 to 65 % in 2009–2010. Antibody prevalence has been declining at a rate of about 2% per year on average. Infection does contribute to the development of certain lymphomas, either by suppressing immune function or by other mechanisms such as chronic inflammatory induction (Thandra et al. 2021).

Method

Detection of membranous latent protein LMP-1 of EBV from paraffin-embedded tissue blocks of patients with Hodgkin disease were done using immunohistochemistry technique. EBV - LMP1 primary antibody, Monoclonal Mouse Anti –Epstein-Barr Virus, LMP-1, Clones CS1-4, Ready-to-Use. The cytoplasmic and membranous staining pattern were done based on DAKO kit. Blueish staining of the cytoplasm and membrane was regarded as negative for EBV LMP-1, whereas brown granular cytoplasmic and membrane staining was viewed as positive for EBV LMP-1. A tissue that was known to have EBV LMP-1 used as positive control. Detection of EBV in the FFPE blocks by polymerase chain reaction (PCR) according to the manufacturer's instructions, Genomic DNA was extracted from tumor tissue by using a FavorPrep DNA FFPE tissue kit (Favorgen,Taiwan). The LMP-1 gene of EBV was amplified by using Hot Start Taq (Bioneer, Korea) with forward primer. ′5- ATCGTGGTCAAGGAGGTTCC -3′ and reverse primer ′5- ACTCAATGGTGTAAGACGAC -3′.209bp Baer et al., 1984. PCR consisted of 30 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 70 °C for 1 min. A lymphoma specimen diagnosed as LMP-1 - positive was used as a positive control. The PCR products were analyzed by electrophoresis in 1% agarose gel (Medhat and Aljanaby, 2022).

Results

Distribution of lymphoma's FFPE block according to gender

Table 1 illustrated the distribution of study sample according to gender, Where the male constitutes 23/40 (59.0%) of Hodgkin lymphoma patients and 16/40 (41.0%) of patients with non- Hodgkin lymphoma. Female consist of 17/40 (41.5%) of Hodgkin lymphoma patients and 24/40 (58.5%) of patients with non-Hodgkin lymphoma. but there is no statistical significance at P= ≤ 0.05.

Table 1: Distribution of lymphoma's FFPE block according to gender

Gender / Disease	Male	Female	Total	P-value
	N (%)	N (%)	N (%)	
Hodgkin lymphoma	23(59.0)	17(41.5)	40(50.0)	0.11
Non- Hodgkin lymphoma	16(41.0)	24(58.5)	40(50.0)	
Total	39(100)	41(100)	80(100)	

Distribution of lymphoma's FFPE block according to age groups

The distribution of patients with Hodgkin and Non-Hodgkin lymphoma according to age group as the current results revealed that the age group of more than 50 years was higher (47.5%) in patients with non-Hodgkin lymphoma in comparison to Hodgkin lymphoma patients (20.0%), in contrast the age group (10-20) was the highest in patients with Hodgkin lymphoma (25.0%) whereas in patients with non-Hodgkin lymphoma constituted (0.0%), there was highly significant at P= ≤ 0. 05. Table (2).

Table 2: Distribution of lymphoma's FFPE block according to age groups

Lymphoma / Age group	Hodgkin	Non-Hodgkin	Total	P-value
	N (%)	N (%)		
<10	1(2.5)	2(5)	3(3.8)	0.001
10-20	10(25)	0(0)	10(12.5)	
21-30	9(22.5)	4(10)	13(16.3)	
31-40	4(10)	10(25)	14(17.5)	
41-50	8(20)	5(12.5)	13(16.3)	
>50	8(20)	19(47.5)	27(33.8)	

Distribution sample for EBV-LMP1-gene according to gender

The results of the present study showed that the EBV- LMP-1 protein was positive in 12.5% of the all blocks (80), 4/40 (10%) of non-Hodgkin lymphoma, while 6/40 (15%) of patients with Hodgkin lymphoma was positive for of EBV- LMP-1 protein, although there was a difference among the two-study group in frequency but

there was no significant difference at p-value ≤ 0.05. There was no statistical difference between male and female for EBV-LMP-1 and the frequency was equal in the two groups, where 5 males are positive of EBV-LMP-1 (3/5 HL) HL and (2/5 NHL) samples, as well as 5 females were positive of EBV-LMP-1 (3/5 HL) HL and (2/5 NHL) samples as in (Table 3).

Table 3: Distribution sample for EBV-LMP1-gene according to gender

Lymphoma / Gender			Gender		Total	P-value
			Male	Female		
Hodgkin	LMP-1	+Ve	3(60%)	3(60%)	6(60%)	0.9
		-Ve	20(58.8%)	14(38.9%	34(48.6%)	
Non-Hodgkin	LMP-1	+Ve	2(40%)	2(40%)	4 (40%)	
		-Ve	14(41.2%)	22(61.1%)	36(51.4%)	
Total	LMP-1	+Ve	5(100%)	5(100%)	10(100%)	
		-Ve	39(100%)	41(100%)	80(100)	

Discussion

The incidence of Hodgkin's disease varies greatly depending on age, gender, race, geographical location, social class, and histological subtype. The EBV is linked to approximately 40% of cases in developed countries. It is well known that the age-incidence curve for Hodgkin diseases in developed countries is bimodal, with the first peak occurring between the ages of 15 and 34 and the second peak occurring in over 60 years (Wang et al. 2019). The results of the current study the age group (20-35) aligned with this line. EBV positivity is common in children (10 years) and older adults (>60 years). Patients with HL. The current results found that HL were more likely to be EBV-associated than NHL cases. The current study's results aligned with (Houldcroft and Kellam 2015) and (Kimura and Fujiwara 2019; Swerdlow et al. 2016) findings that found young children and older people, EBV-positive HL is more prevalent than EBV-negative HL. Within HL cases, age effects were observed, with younger children being more likely to have EBV-associated disease than older. And these results correspond with many other studies(Nagpal, Descalzi-Montoya, and Lodhi 2021)(Jimenez et al. 2021). On other hand, Within NHL cases, age effects were observed, with elderly being more likely to have EBV-associated disease than young(Shimoyama et al. 2008)(Ok et al. 2013). And these results correspond with a study conducted by (Murthy et al. 2017), revealed that the prevalence of EBV-positive in DLCBL ranges from 2.5 to 14.0 %, with East Asians having a higher incidence and the majority of cases occur in patients over the age of 50, with a male predominance. Moreover, other study achieved by (Sun et al. 2022) showed that males and females aged 50–69 had an age-standardized NHL incidence rate of 17.54 and 11.75 per 100,000 individuals, respectively. NHL was the most common type of malignant lymphoma for both males and females globally (from 81 % to 95 % of malignant lymphoma in males, and from 83 % to 96 % of malignant lymphoma in females(Saito and Matsuoka 2020; Alhasnawi and Aljanaby, 2022). The incidence of NHL had increasing rate, particularly among women, the younger population, and people from Asian countries(Huang et al. 2022). Of note, the results of this study showed a proportion of Hodgkin lymphoma (59% in males and 41% in females) and these

results agree with the finding of an Indian population conducted by(Saito and Matsuoka 2020). The result of the current study revealed that EBV-LMP-1 marker found in 12.5% of cases that had been diagnosed by PCR and IHC. There are several reasons why EBV cannot be detected in all cases. Given the sensitivity and range of assays in widespread use, failure to detect genomes with low copy number or that are defective appears unlikely. It's possible that EBV is using a hit-and-run mechanism in 'negative' cases to avoid detection. Regarding Hodgkin lymphoma, the results of present study revealed that 15% of cases were positive for EBV and these results may slightly differ due to sample size and ethnicity. The results of present study regarding non-Hodgkin lymphoma do not agree with finding of studies conducted by ((Wong et al. 2022)(Sung et al. 2021) that revealed NHLs are responsible for more than 75% of all lymphomas, where NHLs are responsible for only 10% of positive cases and this may be attributed to ethnicity variation and sample size (Abdulla et al., 2022).

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