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Detection of Epstin Barr Virus *lmp-1 gene* associated with lymphoma

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Abstract : Epstein-Barr virus (EBV) infection has been associated with the aetiopathogenic mechanisms of several neoplastic and non-neoplastic disorders. Although the precise mechanisms of the tumourigenic actions of EBV have not yet been fully elucidated, this virus has been strongly linked to subtypes of Hodgkin's (HL) and non-Hodgkin's lymphomas (NHL) (especially Burkitt's lymphoma), nasopharyngeal carcinoma and gastric carcinoma, among several others. Patients group included 22 Formalin fixed paraffin embedded (FFPE) blocks were obtained from biopsies collected from theImam Al-Hussein Medical City in Kerbala Province-Iraqas well as many private histopathology laboratories. Polymerase chain reaction (PCR) used to investigate the prevalence oflatent membrane associated protein-1 (LMP-1) gene variants in EBV-positive malignant lymphoid disorders. We identified 22 patients with lymphoproliferative disorders (HL and NHL) with age of 3-75 years (15 males and 7 females). LMP-1 gene was detected in 6(40%) of non Hodgkin lymphoma (NHL) patients compared with 9 (60%) for those with LMP-1-negative tumors. We concluded that Epstein-Barr virus (EBV)LMP-1 gene can be associated with most non-Hodgkin's lymphomas (NHL) cases.
Keywords: Epstein-Barr virus, LMP-1 gene, lymphoma (HL and NHL).

Introduction:

Lymphoma is a cancer of the white blood cells, namely lymphocytes, that happen to constitute the lymphatic system. The two main types of lymphoma are Hodgkin lymphoma and non-Hodgkin lymphoma. Lymphoma is the most common blood cancer and the third most commoncancer of childhood. Lymphoma occurs when lymphocytes,a type of white blood cell, grow abnormally. The body hastwo types of lymphocytes: B lymphocytes, or B-cells, and Tlymphocytes, or T-cells. Although both cell types can develop into lymphomas, B-cell lymphomas are more common. Likenormal lymphocytes, those that turn malignant can grow in many parts of the body, including the lymph nodes, spleen, bone marrow, blood or other organs[(4)]. Non-Hodgkin lymphoma is a cancer that starts in cells called lymphocytes, which are part of the body's immune system. Lymphocytes are in the lymph nodes and other lymphoid tissues (such as the spleen and bone marrow)[(1).]

Hodgkin's Lymphoma (HL) is a particular type of cancer within the larger group of lymphomas. This cancer develops and affects the immune system, which is responsible for protecting the body from certain diseases and infections. The incidence of HL continues to rise and most often affects younger adults and males and is the most frequently occurring cancer among 15 to 34 year olds in industrial nations[(5)]. There are a few distinct differences between HL and NHL including how the disease spreads, where tumors are most commonly found in the body and variances in symptomology experienced by individuals. In addition, treatment protocols are very different. HL is not as common as NHL and the age of onset for HL occurs in a bimodal distribution

with the average age of onset at 28 years and a less substantive peak after age 55, whereas it is less common to see cases of NHL in people under age 50 [6]. For both HL and NHL the most common location of the tumors is in the lymph nodes [7].

Specific to HL, malignancies are also found in the chest area, whereas in NHL tumors inthe abdomen are more common. Similarly, in HL as few as 4% of cases demonstrate cancer outside the lymph nodes, which differs significantly in NHL where nearly one quarter of all patients have confirmed lymphoma outside the lymph nodes. An important difference between both lymphomas surrounds the progression of disease. In HL, the progression is often quite orderly spreading in a downward pattern from the initial site to each lymph node and rarely diagnosed in stage IV. Additionally, when HL first presents below the diaphragm it most frequently progresses to the spleen. Conversely, in NHL nearly 40% of diagnosed cases are at stage IV, which are more likely to spread and not as predictable in terms of their progression[7].

Follicular lymphoma (FL) is the most common indolent lymphoma and the second most common non-Hodgkin lymphoma – accounting for about 10-20% of all lymphomas in Western countries. The incidence of FL, as with other non-Hodgkin lymphoma, is rising, although varies between geographical regions and ethnic groups being lower in Asian and sub-Saharan African countries than in western regions likely as a combination of both genetic and environmental factors[2],[3].In addition many factors are responsible for cell transformation of lymphoma and some of the most important ones are viruses[8].

Viruses act in inducing cancers through different methods. Transforming retroviruses, for instance, carrycellular genes which are transcribed by the virion-associated reverse transcriptase, followed by integration of the double stranded DNA copy into the chromosomal DNA of the cell [9],[10]. Several different mechanisms are encountered before cancers are eventually formed. Epstein Barr Virus (EBV or Human herpesvirus4) belongs to the genus *Lymphocryptoviridae*, thegamma1 subtype of the subfamily *Gammaherpesvirinae* and is one of the most common viruses in humans. It is present in all populations, infecting more than 95% of all individuals within the first four decades of life. This virus has been demonstrated to be involved in the development of many malignancies with the list of such malignancies progressively increasing. The first association was with the endemic Burkitt's lymphoma. Subsequently, other lymphomas(subtypesof both Hodgkin's and non-Hodgkin's lymphomas) a real so now known to be associated with EBV infection. Epithelial malignancies such as lymphoepitheliomas of nasopharynx and stomach are currently included in the list of EBV associated tumours[8].

EBV infects resting B cells and turns them into continuously proliferating lymphoblastoid cell lines that express nine latency-associated viral proteins, including six nuclearantigens (Epstein-Barr nuclearantigen(EBNA)-1, -2, -3A, -3B, -3C and LP) and three membrane proteins (latent membrane protein (LMP)- 1, -2A and -2B) [11].The aim of this study was to investigate the hypothesis that EBV is causatively associated with all, or most ,lymphoproliferative disorders cases. The results provide good evidence that some patients with NHL have been infected with EBV, thus EBV can be associated with NHL cases.

Methods:

Formalin fixed paraffin embedded (FFPE) biopsy tissue blocks from nodal and extranodal sites that were obtained from patients who had undergone surgical operation done from them . Study groups included 22 FFPE blocks were obtained from biopsies collected from theImam Al-Hussein Medical City in Kerbala Province-Iraqas well as many private histopathology laboratories. These blockes includes 15 non Hodgkin lymphoma (NHL) 8 male, 7 female, Hodgkin lymphoma (HL) were 3 males, and 4 males with reactive follicular hyperplasia. The ages of patients with lymphoproliferative disorders (NHL and HL) were 3-75years.The patients with lymphoproliferative disorders enrolled in this study were 15 males and 7 females.

DNA extraction:

Genomic DNA was extracted from sections of FFPE blocks by using Purelink Genomic DNA Mini kit (Invitrogen) (3).

For EBV DNA analysis, Polymerase Chain Reaction technique was used. EBV *lmp-1* gene were detected by PCR- amplification performed in a thermal cycler PCR system. Primers were shipped in a

lyophilized state. The sequences of primers (EBV *lmp-1* gene) were listed in table (1). Bioneer 100 bp DNA ladder was used in this study.

Table (1): Sequence of primers used for PCR amplification of EBV *lmp-1* gene

Type of disease	Number	Positive	Nagative
NHL	15	6 (40%)	9 (60%)
HL	3	0 (0.0%)	3 (100%)
RF	4	0 (0.0%)	4 (100%)

Results:

The EBV *lmp-1* detected by conventional PCR in all tissues of studied groups. The positive results (40%) of EBV *lmp-1* was shown among 6 out of 15 NHL cases, while (0.0%, 0.0%) was shown in HL and reactive follicular hyperplasia respectively. The result of amplification of DNA samples of EBV *lmp-1* with selected forward and reverse primer was 190 bp band as in figure **(1).**

Table (2) Frequency of EBV *lmp-1* in studied groups

primer	sequence	Product size	reference
forword	**5'- ATTTATTTTTGCTTGCCATT -3'**	**190bp**	**202-203**
reverse	**5'- GTCTGTCTGTCTGTCCGTCA -3'**		

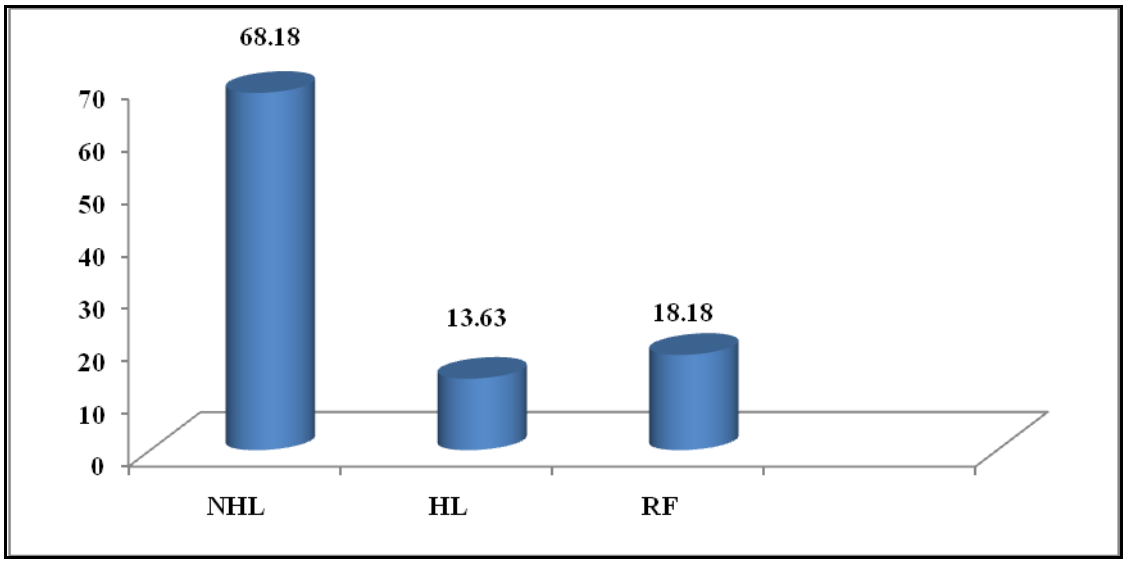

Figure (1): Total number of studied groups (expressed as %) *NHL: non Hodgkin lymphoma, HL: Hodgkin lymphoma, RF: Reactive follicular lymphoma

***NHL: non Hodgkin lymphoma, HL: Hodgkin lymphoma, RF: Reactive follicular lymphoma**

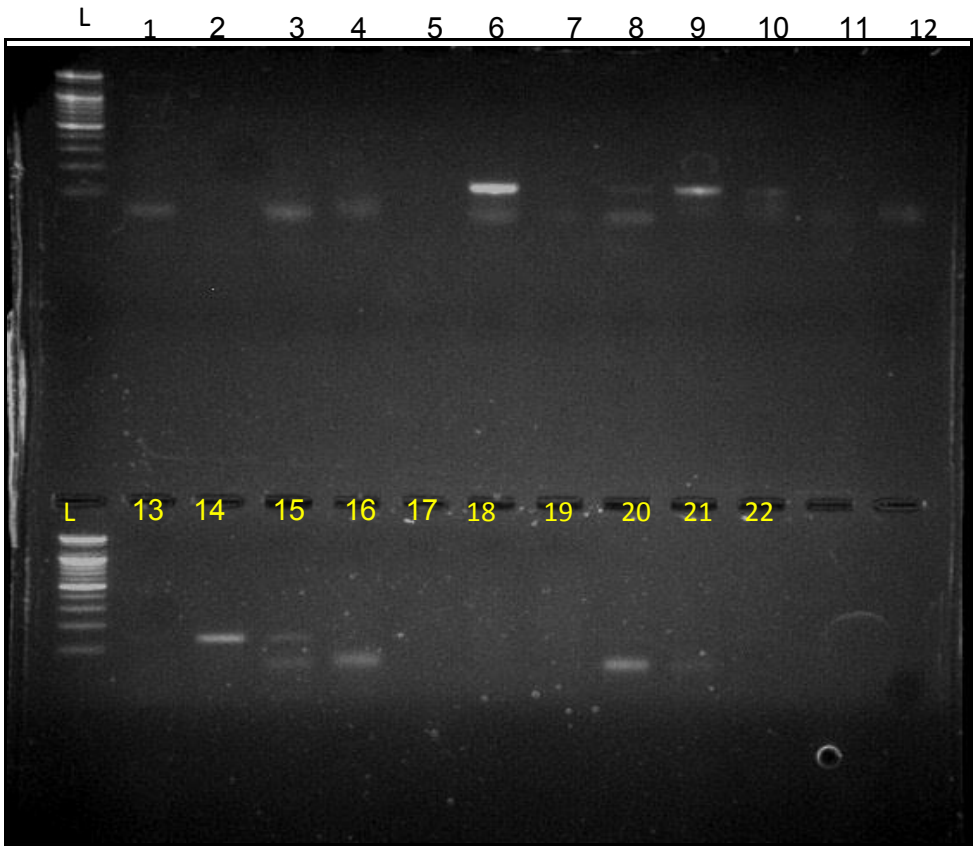

Figure (2): The PCR amplification products of the EBV *lmp-1* on ethidium bromide stained agarose gel (2%), 70 volt for 1 hour. the size region 190 bp are indicative positive result for EBV *lmp-1*.
Lane (L):100 bp DNA ladder
A- Lane (6, 8, 9, 10, 14, 15) positive result in NHL.
B- Lane (17, 18, 19) nagative result in HL, (20, 21, 22) negative result in reactive follicular hyperplasia.

Discussion:

The fact that the detection of raised levels of EBV preceded the development of Hodgkin lymphoma (HL) by several years supported the growing suspicion that EBV contributed to the carcinogesis in HL.Ocheni *et al.* (2010) reported that, following infectious mononucleosis, the relative risk of developing HL compared to individuals without prior history ranged between 2.0 and 5.0[(23)].

In addition, that EBVDNA was detected in 20-25% of HL tumor specimens. The association between EBV and HL is less, with percentages of between 20% and 50% for North American and European cases, 57% for China but much higher rates in underdeveloped countries such as Peru and Kenya. In spite of the increased knowledge about the contributory roles of the various carcinogenic mechanisms in HL,the precise contribution of EBV remains yet to be fully understood. In particular, it is very important to identify the roles of latent virus products, particularly LMP-1 and LMP-2[(23)].The present study agreement with a study of Gallagher *et al*. that identify a case of HL in which no evidence of defective EBV genomes in this case. Also analysis of a further 30 cases provided no evidence that defective EBV genomes are a feature of HL, thus EBV cannot be associated with all cases of HL[(21)].

Moreover,LMP1, the major transforming protein of EBV, can up-regulate the Bmi-1oncogene and that the up-regulation of Bmi-1 in EBV-positive and EBV-negative HL cells is mediated by nuclear factor(NF)-$_k$B[(19)].Bmi-1 up-regulated a number of genes and which are also known transcriptional targets of LMP1.[(17),(18),(20)]. Dutton*et al*.,2007 suggest that Bmi-1 contributes to LMP1inducedoncogenesis in HL[(16)].

Furthermore, a number of studies have demonstrated the association between EBV infection and several subtypes of NHL[12]**.** A higher prevalence of EBV in lymphomas diagnosed in children younger than 10 years old in pediatric HL and NHL [13),(14)].cohen *et al.,* 2013 found that LMP1 expression localized at the cytoplasm and surface membrane of neoplasticcells was positive in (60%) EBVsamples[15].Also a study in African children, which analyzed series of NHL that included a subset of diffuse large B-cell lymphoma (DLBCL), and described an association of 43–44.4% between EBV and this lymphoma subtype[22].

Conclusion:

Epstein-Barr virus infection has been associated with the pathogenesis of several cancers. Recent studies indicates the role of EBV in the epigenetic alterations in several types of cancers including gastric carcinoma, nasopharyngeal carcinoma, and lymphomas.

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