Article
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol.31; No.3.| 2023

PCR-Based Study for Molecular Documentation of Human Herpes Virus Type – 8 and P15 Polymorphism in a Group of lymphoma Patients.

Ameer J. Abbas Al-Jawdhari[1*], Shakir H. Mohammed Al-Alwany[2]

1 College of Science, University of Babylon, ameeralq@gmail.com, Hilla, Babylon, Iraq.
2 College of Science, University of Babylon, shakir_immuno@yahoo.com, Hilla, Babylon, Iraq.
***Corresponding author email:** ameeralq@gmail.com; mobile: 07806271145

دراسة معتمدة على استخدام تقنية تفاعل البلمرة المتسلسل للتوثيق الجزيئي لتعدد الأشكال الوراثية في فيروس الهربس البشري من النوع الثامن والجين المثبط لنمو الخلايا السرطانية لمجموعة من المرضى المصابين بمرض سرطان الغدد اللمفاوية

امير جاسم عباس عبيد[*1]، شاكر حماد محمدالعلواني[2]
1 - كلية علوم الحياة، جامعة بابل، ameeralq@gmail.com بابل، العراق
2- كلية علوم الحياة، جامعة بابل shakir_immuno@yahoo.com بابل، العراق

Accepted: 13/7/2023 **Published:** 30/9/2023

ABSTRACT

Background:

Since its initial identification with primary effusion lymphoma (PEL), the range of lymphoproliferative illnesses associated with human herpesvirus 8 (HHV-8) infection has steadily expanded. Systemic B cell lymphoma often has p16(INK4a) and p15(INK4b) inactivation, which may indicate a poor prognosis. 200 specimens are studied cross-case-control. Lymphoma (LY) patients and normal people (control group) are sampled. Iraq's Middle Euphrates region's general hospitals and private clinics provided the samples. The study included 17-75-year-olds. Samples were collected between October 2022 and February 2023. PCR-based sequencing detected HHV-8 and p15 gene polymorphisms.

Materials and Methods:

PCR was used to detect HHV-8 and P15 gene polymorphism in 100 patients and 100 healthy individuals. Conventional PCR sequenced HHV-8 and p15 gene polymorphisms

Results:

31 out of 64 (48.4%) specimens tested positive for HHV-8, while 33 out of 64 (51.6%) tested negative. The age group (56-75 years) has 48% of HHV8-infected lymphomas, while the age groups (15-35 years) and (36-55 years) had 23% and 29%, respectively. The P15 polymorphism shows that the AA genotype increased lymphoma, while the TT genotype decreased it ($p<0.05$).

Conclusion:

Despite the small sample size, our findings suggest that HHV-8 and p15 gene polymorphism may affect lymphocyte tumor biology and development.

Key words: HHV-8; Lymphoma; PCR; P15; polymorphism; sequencing.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

INTRODUCTION

A variety of lymphoid neoplasms have omit connected to the advent of human herpesvirus type 8 (HHV8), a gamma herpesvirus. including diffuse large B-cell lymphoma that is HHV8 positive and not otherwise specified, multicentric Castleman disease, and germ inotropic lymphoproliferative disorder [1-4]. Additionally, to these well-defined elements associated with HHV8, rare instances with unusual and overlapping characteristics, Epstein-Barr virus (EBV) coinfection, and reactive lymphadenitis associated with HHV8 have all omit reported [5–10]. The significance of these cases in therapeutic practice, however, is unclear. Most HHV8-positive lymphoproliferative diseases (LPD) also manifest in the context of immunosuppression, which complicates both diagnosis and treatment [11].

The significance of cyclin P15 in carcinogenesis has gained more attention as of late. Multiple studies have revealed that downregulation of its expression contributes to cell cycle dysregulation and unchecked cell proliferation in cancer cells [12].

The strong response to local treatment and positive prognosis of primary cutaneous B cell lymphomas set them apart from systemic lymphoma, another kind of lymphoproliferative illness. A poor prognosis may be connected with the frequent observation of p15(INK4b) and p16(INK4a) inactivation in systemic B cell lymphoma. However, there have been no systematic analyses of primary cutaneous B cell lymphomas. Loss of heterozygosity, homozygous deletion, hypermethylation of the promotor region, and point mutations are all ways in which p15/p16 might be rendered inactive [13]. This study is designed to estimate the polymorphism of the P15 gene and the percentage of HHV-8 infection in a group of Iraqi patients with lymphoma in the Mid-Euphrates Sector of Iraq.

MATERIALS AND METHODS

This study is set up as a case-control study.

A. Study age groups

Blood from each study excluded and included criteria of Patients with the NHL enroll, is classified into: -

1. A group of blood specimens (NO :100) from Patients with the NHL.
2. Blood specimens (NO :100) of apparently healthy persons as a control group.

B. Sample Collection

Blood samples will be gathered from individuals diagnosed with the NHL (Non-Hodgkin's lymphoma) as well as from apparently healthy individuals serving as the control group. Five ml of blood sample (2ml EDTA Blood and 3ml in gel tube) were collected and then viral genome and total DNA were obtained and stored at -20°C/-80°C till used.

The samples will be collected from both general hospitals and various private clinics located in Middle Euphrates, Iraq such as the hospitals in Babylon (Mirgian and Imam AL-Sadiq Teaching Hospital) and Baghdad (The City of Medicine, Baghdad Teaching Hospital, and National Center for Educational Laboratories).

ISSN: 2312-8135 | Print ISSN: 1992-0652

info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 7/17/1336 (including the number M220904 and the date in 28/9/2022) to get this approval.

Sequencing of PCR Products

The phrase "DNA sequencing" refers to procedures for figuring out the arrangement of the nucleotide bases (adenine, guanine, cytosine, and thymine) in a DNA according to Early in the 1970s, 2-dimensional chromatography-based laboratory techniques are initially used by academic researchers to obtain the first DNA sequences. DNA sequencing has grown more effective and quicker thanks to improvements in dye-based sequencing techniques and automated analysis. It has become essential for basic research on biological processes to understand the DNA sequences of genes and other genomic regions, and this knowledge has implicated in many fields, including diagnostics and forensics.

Total DNA Extraction

DNA extraction kits (Intron / Korea, CAT.NO= 14001) were used to extract total genomic DNA from the blood samples of both the patients and the control groups.

1. Viral DNA Extraction

Using a viral DNA extraction kit, viral genomic DNA is retrieved from blood patients and control groups.

2. Evaluation of extracted DNA concentration and purity

The quantity and purity of DNA can be determined by (Nano drop) at the absorbance at 260 nm and 280 nm, respectively. (Nano drop) at the absorbance at 260 nm and 280 nm respectively can determine the DNA quantity and purity.

PCR Conditions and primers

Primers sets used in this study to detect the HHV-8, and SNP P15 polymorphism with their product size and source as well as origin are listed in (Table1). The Promega (USA) manufactured of PCR primers used in this work.

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

Table 1. PCR primers and their conditions used in this study, Promega (USA).

Primer		Sequence (5----------›3)	Amplicon size (bp)	Conditions		Cycle No.	Source/origin
HHV-8	F	CAGTCTGGCGGTTTGCTTTC	**592bp**	ID	95˚C / 5min	40	IDT / USA
				D	95˚C / 1min		
	R	GTAGGTGCGGTTGCAAATGT		A	**53˚C / 45sec**		
				E	72˚C / 1min		
				FE	72˚C / 5min		
P15	F	ATGGAGCTAGAAGCAGGACT	**501bp**	ID	95˚C / 5min	40	IDT / USA
	R	CTCCCATTTGGTTTATAAAATCCCT		D	95˚C / 1min		
				A	**50˚C / 45sec**		
				E	72˚C / 1min		
				FE	72˚C / 5min		

Abbreviations: ID, Initial Denaturation; D, Denaturation; A, Annealing; FE, Final Extension; F, Forward primer; R, Reverse primer.

Preparation of Reaction Mixture

Standard thermal cycler (Biometra, Germany) was used to do the PCR amplification. About (4 µl) of sample DNA is placed in each of the tubes containing the PCR master mix. Each PCR master mix tube received (2 µl) of forward and reverse primers. As shown in (Table 2), PCR master mix tubes were refilled with distilled nuclease-free water to a final volume of (25 µl).

Table 2. AccuPower® PCR tubes: concentration and volume guidelines for performing PCR.

No.	Content of PCR Reaction Mixture	Volume/ µl
1	Master mix	12 µl
2	Forward primer	2 µl
3	Reverse primer	2 µl
4	Sample DNA	4 µl
5	Nuclease free water	5 µl
Total		**25 µl**

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

Agarose Gel Electrophoresis

Robinson and Lafleche (2000)'s protocol was followed for the agarose gel electrophoresis. Total DNA extracts and PCR products were utilized to detect the viral genome using this method .

1.5% agarose gels were stained with 5 l of Red Safe for 1 hour at 85 volts to run PCR products. Specifically, 5 liters of amplification products and 1 liter of loading dye were poured into the gel well. Amplified gene electrophoresis fragments were measured using the 100-1500 bp DNA marker (Intron.S/Korea). Gel documentation system (Biometra-Germany) is used to photograph the DNA bands [17].

Statistical Analysis:

The Chi-square test is used to determine the relationship between the analyzed variables in this study, and all statistical analyses were performed using the SPSS program, Version 24, with a p value of 0.05 being considered significant.

RESULTS AND DISCUSSION;

I. Results:

1) Age distribution of study groups:

The studied blood specimens are related to patients with lymphoma whom their age ranged from (17 to 75) years (mean = 49.92 + 11.5 years), while their control counterparts have a mean of 33.5 + 13.4 years. However, on comparing the age of these two groups, no significant variations were detected (P > 0.05) (Table 3).

Table 3. The age of patient's lymphoma.

Studying Groups	N	Mean Age	S.D	S.E	Min	Max
Lymphoma	100	49.92	11.5	2.2	17	75
Control	100	33.5	13.4	2.7	20	68
Statistical Analysis	Non-significant (P > 0.05) = 0.09					

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

2) The Sex of the studied patients with Lymphoma:

The male to female ratio in our samples for lymphoma is 1.75:1, with the male gender making up 36% and the female gender 64%. In the control group, the male gender made up 65% and the females 35%. In statistical analysis, there is no significant difference between the lymphoma patient and control group (greater than P 0.05) (Table 4).

Table 4. Distribution of study groups according to their gender

Sex	Lymphoma		Control		P-value
	No.	%	No.	%	
Male	36	36	35	35	0.4
Female	64	64	65	65	
Total	100	100	100	100	

3) Distribution of the studied patients according to their age strata and Sex:

Regarding the age of lymphoma cases, 24 /100 (24 %) of them were between the age of 15-35 years (12 men and 12 women), 29 / 100 (29 %) are between the age of 36-55 years (12 men and 17 women) and 47 / 100 (47 %) were between the ages of 56-75 years (12 men and 35 women). The highest male and female frequencies (12 and 35 respectively) were found in the age group of those 56–75-years (Table 5).

Table 5. The categorization of individuals diagnosed with Lymphoma based on their age groups and Sex.

Age Stratum of Lymphoma Patients	Sex		Total	
	Male	Female		
	No.	No.	No.	%
15-35	**12**	**12**	**24**	**24**
36-55	**12**	**17**	**29**	**29**
56-75	**12**	**35**	**47**	**47**
Total	**36**	**64**	**100**	**100**

4) Detection Rates of HHV-8 by Using PCR Technique

I. Extraction of Viral Genome

Regarding the detection of viral DNA/RNA extraction kit to extract nucleic acid, it is found that 31 out of 64 (48.4%) of the specimens are containing the viral genome (Figure 1). In contrast to the control group, 5 out of the (5%) post-mortem specimens were having viral nucleic

ISSN: 2312-8135 | Print ISSN: 1992-0652 | www.journalofbabylon.com | jub@itnet.uobabylon.edu.iq | info@journalofbabylon.com

acid (Table 6). A statistically significant difference (p = 0.02) is seen between the two groups' results.

Table 6. Viral genome detection in blood specimens

Viral Genome		Lymphoma	AHC group +
Positive	N	31	5
	%	48.4%	5%
Negative	N	33	95
	%	51.6 %	95%
Total	N	64	100
	%	100%	100%

+AHC means apparently health control

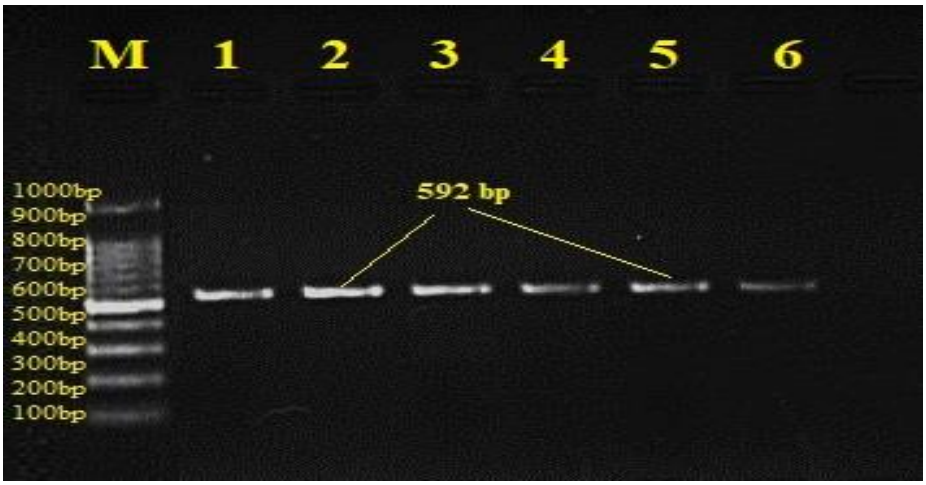

Figure 1: Extraction of HHV-8 genome from Blood specimens, 1.5% Agarose Gel Electrophoresis, TBE 1X, at 85 Volt for 1hour.

II.HHV-8 Genome Detection Using Conventional PCR:

According to PCR detection results, 48.4% (31 out of 64) of the specimens have HHV-8 genome, while 51.6 (33 out of 64) specimens show negative results for HHV-8 genome detection, and as indicated in (Table 7) and figure (2). The statistical analysis of the differences between these 2 groups are significant (p = 0.03).

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Table 7. The PCR results of HHV-8 DNA in Blood specimens of study groups.

	Lymphoma N/ %	AHC* N/ %
Positive	**31 (48.4%)**	**5(5%)**
Negative	**33 (51.6%)**	**95(95%)**
Total	**64 (100%)**	**100 (100%)**

AHC* mean apparently health control

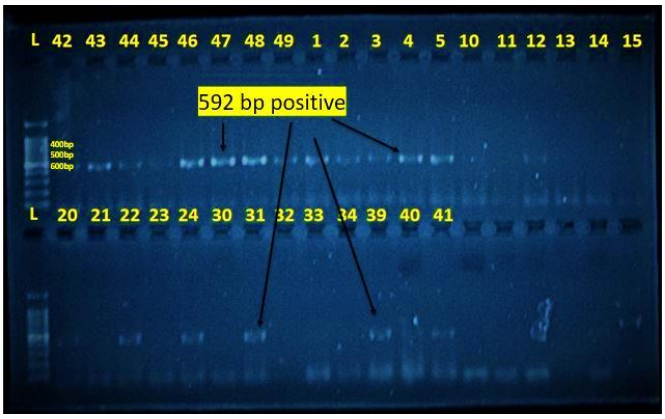

Figure 2: HHV-8 PCR product (592 bp) presence and absence in several specimens. Electrophoresis of PCR products was used to separate them in a 1.5% agarose gel for 1 hour at 85 V/Cm. M: 1500 bp long DNA ladder for markers.

III. The HHV-8 Results in Blood specimens according to the Age Groups.

The age strata (15- 35 years), (36-55 years), and (56-75 years) each accounted for **23%**; **29%** and **48%**, respectively significant differences ((P<0.05)) are found when these age groups were compared statistically (Table 8).

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Table 8. Frequency of HHV-8 PCR results among patients with lymphoma according to their age strata

Age Stratum	Years	HHV-8			P value
		No.	Positive	Negative	
	15-35	15	6	9	Anova test P=0.04 S. (P<0.05)
		23%	40%	60%	
	36-55	18	8	10	
		29%	44.44%	55.56%	
	56-75	31	17	14	
		48%	54.84%	45.16%	
Total		64	31	33	
		%100	48.44%	51.56%	

IV. Detection of SNPs *P15* Polymorphisms by PCR

Table (9) and Figure (3) shows the positive results of SNPs *P15* polymorphisms by PCR detection in patients with lymphoma infection as well as apparently healthy control groups. When compared to control groups, lymphoma prevalence is significantly higher in the AA genotype and significantly lower in the TT genotype ($p< 0.05$).

Table 9. The positive results of SNPs *P15* polymorphisms by Sequencing.

SNP *P15* T>C	Patients with Lymphoma *N (100)*		AHC *N (100)*	
Genetic model	*N (%)*	P	*N (%)*	P
T\T	10 (13.3)	<0.05	10 (40)	<0.05
T\A	32 (42.7)	>0.05	20 (80)	<0.05
AA	33 (44)	<0.05	5 (20)	<0.05

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

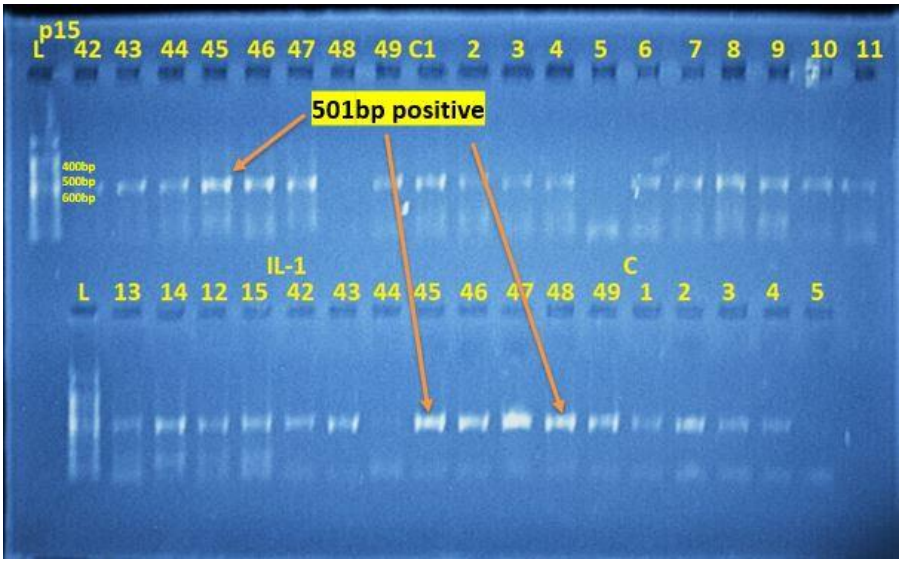

Figure 3: P15 PCR product (501bp) presence and absence in several specimens. Electrophoresis was used to separate the PCR products in a 1.5% agarose gel for 60 minutes at 85 V/Cm. M: 1500 bp long marker DNA ladder.

II. DISCUSSION:

Primary effusion lymphoma (PEL) predominantly impacts individuals who have a significantly compromised immune system, particularly individuals living with HIV. There have been a few instances where PEL has omit observed in individuals with conditions such as malignancy, cirrhosis, or those who have undergone organ transplantation [14-15]. The uncontrolled growth of cells infected with HHV8 is associated with the onset of the disease [16].

HHV8, a gamma herpesvirus, exhibits varying prevalence depending on geographic regions and specific exposures. It is frequently found in equatorial areas, such as Africa and South America, where more than 50% of the population has omit exposed to the virus. The occurrence of the condition in the Mediterranean area, Middle East, and the Caribbean is moderate, with rates ranging from 5% to 20%. However, in North America, Northern Europe, and Asia, the virus has a low prevalence, affecting less than 5% of the population [14]. In the same regions, men exhibit a notably higher seroprevalence compared to women. The main mode of transmission for HHV8 is saliva, but it can also transmit to a lesser extent through injection drug use, blood transfusions, and organ transplantation. Among various population groups in the United States, HIV-positive men who have sex with men face the highest risk, with seroprevalence rates reaching as high as 30%. Additionally, transplant patients have an estimated seroprevalence of 15%. [14]. Available evidence supports the observation that the transmission of HHV8 through a solid organ

ISSN: 2312-8135 | Print ISSN: 1992-0652

info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

transplant is substantiated [15,17]. Patient of the recent study did not possess any identified risk factors for HHV8. Among the limited number of cases reported in transplant recipients, most have been observed in regions where HHV8 is more prevalent compared to the United States. Given the information provided, there is a reasonable possibility that the HHV8 in this particular situation might have originated from the donor and been transmitted through the transplanted organ. However, it is not feasible to obtain conclusive verification Polymorphisms.

Several studies [18-19] examined the methylation levels of P15 and P16 in lymphoma and leukemia specimens. In a study of 56 instances of non-Hodgkin's lymphoma, researchers observed that aberrant methylation of the P15 and P16 genes was very prevalent in both B and T cell lymphomas, particularly in high-grade illness [18]. Further analysis of 64 cases of acute leukemia revealed that genes P15 and P16 are methylated in 40% and 42% of cases, respectively, with low rates of mutation and deletion [20]. According to Julia et al [21], methylation of the P15 gene is more common than methylation of the P16 gene (45% vs. 29%). P15 and P16 promoter methylation was shown to be selective in 29% and 12% of patients, respectively, whereas both genes were methylated in 17% of patients. In 42% of individuals with Sezary syndrome, the P15 gene was shown to be selectively methylated, but the P16 gene promoter remained unaltered.A small study by Navas et al. [22] of nine patients with patch/plaque and tumor stage mycosis fungoides find that hypermethylation occurred in three of nine plaque samples and allelic loss occurred in one, while hypermethylation occurred in five of nine tumor samples and allelic loss occurred in three of nine tumor samples. No single-point mutations are identified.

Acknowledgments:

The authors are grateful to the staff at the hospitals in Babylon (Mirgian and Imam AL-Sadiq Teaching Hospital) and Baghdad (The City of Medicine, Baghdad Teaching Hospital, and National Center for Educational Laboratories) in Iraq for providing clinical specimens and other resources for the study.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Conflict of interests.

There are non-conflicts of interest.

References

[1] G. Cathomas, "Kaposi's sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 (HHV-8) as a tumour virus", *HERPES-CAMBRIDGE-*, vol. 10, no. 3, pp. 72–77, 2003.

[2] J. Stebbing, L. Pantanowitz, F. Dayyani, R. J. Sullivan, M. Bower, and B. J. Dezube, "HIV-associated multicentric Castleman's disease", *The Journal of the American Society of Hematology*, vol. 83, no. 6, pp. 498–503, 2008.

[3] E. J. Ariza-Heredia and R. R. Razonable, "Human herpes virus 8 in solid organ transplantation", *Transplantation*, vol. 92, no. 8, pp. 837–844, 2011.

[4] S. H. Swerdlow *et al.*, "World Health Organization classification of tumours of haematopoietic and lymphoid tissues", vol. 2. *International agency for research on cancer Lyon, France*, 2008.

[5] E. L. Courville, A. R. Sohani, R. P. Hasserjian, L. R. Zukerberg, N. L. Harris, and J. A. Ferry, "Diverse clinicopathologic features in human herpesvirus 8–associated lymphomas lead to diagnostic problems", *American journal of clinical pathology*, vol. 142, no. 6, pp. 816–829, 2014.

[6] R. M. Seliem *et al.*, "HHV-8+, EBV+ multicentric plasmablastic microlymphoma in an HIV+ Man: the spectrum of HHV-8+ lymphoproliferative disorders expands", *The American journal of surgical pathology*, vol. 31, no. 9, pp. 1439–1445, 2007.

[7] B. Gonzalez-Farre *et al.*, "HHV8-related lymphoid proliferations: a broad spectrum of lesions from reactive lymphoid hyperplasia to overt lymphoma", *Modern Pathology*, vol. 30, no. 5, pp. 745–760, 2017.

[8] J. A. Ferry, A. R. Sohani, J. A. Longtine, R. A. Schwartz, and N. L. Harris, "HHV8-positive, EBV-positive Hodgkin lymphoma-like large B-cell lymphoma and HHV8-positive intravascular large B-cell lymphoma", *Modern Pathology*, vol. 22, no. 5, pp. 618–626, 2009.

[9] S. Sanchez *et al.*, "HHV8-positive, EBV-positive Hodgkin lymphoma-like large B cell lymphoma: expanding the spectrum of HHV8 and EBV-associated lymphoproliferative disorders", *International Journal of Hematology* , vol. 112, pp. 734–740, 2020.

[10] T. Bhavsar *et al.*, "KSHV-and EBV-associated germinotropic lymphoproliferative disorder: new findings and review of the literature ", *The American journal of surgical pathology*, vol. 41, no. 6, p. 795, 2017.

[11] C.-Y. Liu, B.-J. Chen, and S.-S. Chuang," Primary effusion lymphoma: a timely review on the association with HIV, HHV8, and EBV", *Diagnostics*, vol. 12, no. 3, p. 713, 2022.

[12] Han, Y., Kim, J., & Kim, D. "The cyclin-dependent kinase inhibitor p16INK4a regulates cancer stem cell proliferation by modulating cyclin P15 stability" ,*Cancer Research* ,vol .81, no.7, pp.1787-1797, 2021.

[13] F. J. Child, J. J. Scarisbrick, R. Russell-Jones, S. J. Whittaker, E. Calonje, and G. Orchard, "Inactivation of tumor suppressor genes p15INK4b and p16INK4a in primary cutaneous B cell lymphoma", *Journal of investigative dermatology*, vol. 118, no. 6, pp. 941–948, 2002.

[14] E. J. Ariza-Heredia and R. R. Razonable, "Human herpes virus 8 in solid organ transplantation", *Transplantation*, vol. 92, no. 8, pp. 837–844, 2011.

[15] M. Luppi *et al.*, "Molecular evidence of organ-related transmission of Kaposi sarcoma–associated herpesvirus or human herpesvirus-8 in transplant patients", *Blood, The Journal of the American Society of Hematology*, vol. 96, no. 9, pp. 3279–3281, 2000.

[16] R. G. Nador *et al.*, "Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus", 1996.

[17] A. Testa *et al.*, "Fatal sclerosing peritonitis associated with primary effusion lymphoma after liver transplantation: a case report", in *Transplantation proceedings*, Elsevier, 2010, pp. 3849–3853.

[18] A. S. Baur, P. Shaw, N. Burri, F. Delacrétaz, F. T. Bosman, and P. Chaubert, "Frequent methylation silencing of p15 INK4b (MTS2) and p16 INK4a (MTS1) in B-cell and T-cell lymphomas", *Blood, The Journal of the American Society of Hematology*, vol. 94, no. 5, pp. 1773–1781, 1999.

[19] B. Martinez-Delgado *et al.*, "Hypermethylation of a 5' CpG island of p16 is a frequent event in non-Hodgkin's lymphoma", *Leukemia*, vol. 11, no. 3, pp. 425–428, 1997.

[20] S.-X. Guo *et al.*, "Hypermethylation of p16 and p15 genes and RB protein expression in acute leukemia", *Leukemia research*, vol. 24, no. 1, pp. 39–46, 2000.

[21] J. J. Scarisbrick *et al.*, "Frequent abnormalities of the p15 and p16 genes in mycosis fungoides and sezary syndrome", *Journal of investigative dermatology*, vol. 118, no. 3, pp. 493–499, 2002.

[22] I. C. Navas *et al.*, "p16INK4a gene alterations are frequent in lesions of mycosis fungoides", *The American journal of pathology*, vol. 156, no. 5, pp. 1565–1572, 2000.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com
مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

الخلاصة

المقدمة:

منذ بداية العلاقة المسببة الأولية لفيروس التهاب الغشاء اللمفاوي الأولي، توسعت نطاق الأمراض الورمية اللمفاوية المتعلقة بعدوى فيروس الهربس البشري النوع الثامن بشكل مستمر في الورم اللمفاوي خلايا B النظامية، يتم ملاحظة غالبًا عدم نشاط p16(INK4a) و p15(INK4b) وقد يشير ذلك إلى توقع سيء للنتائج. تهدف هذه الدراسة إلى تقدير التشابه الجيني لجين المثبط لنمو الخلايا السرطانية ونسبة عدوى فايروس الهربس البشري النوع الثامن في مجموعة من المرضى العراقيين المصابين بمرض سرطان الغدد اللمفاوية في منطقة الفرات الاوسط في العراق.

طرق العمل:

وفي هذه الدراسة، استخدمت تقنية تفاعل البلمرة المتسلسل للكشف عن وجود تعدد الاشكال الوراثية لفايروس الهربس البشري النوع الثامن وتعدد الاشكال الوراثية في الجين المثبط لنمو الخلايا السرطانية في مئة عينة من المرضى المصابين بمرض سرطان الغدد اللمفاوية ومئة عينة من الأشخاص الاصحاء.

النتائج:

وفقًا لاختبار الكشف بواسطة تفاعل البلمرة الجزيئية المتسلسل، كشفت الدراسة ان 31 من أصل 64 (48.4٪) عينة نتائج إيجابية لفيروس HHV-8، بينما كانت 33 من أصل 64 (51.6٪) العينات تظهر نتائج سلبية لفيروس الهربس البشري النوع الثامن . أما الأورام اللمفاوية التي كانت الأكثر إصابة بفيروس HHV-8 فترتبط بفئة العمر (56-75 عامًا)، حيث بلغت نسبتها 48٪، بينما بلغت نسبة الفئة العمرية (15-35 عامًا) و(36-55 عامًا) على التوالي 23٪و29٪. أظهرت نتائج تحليل تعدد الاشكال الوراثية للجين المثبط لنمو الخلايا السرطانية زيادة ملحوظة في التغاير الجيني للجينات AA وTA، بينما أظهرت التغاير الجيني للجين TT انخفاضًا ملحوظًا في حالات الأورام اللمفاوية مقارنة بمجموعات السيطرة ($p < 0.05$).

الاستنتاجات:

بغض النظر عن قلة عدد العيينات ، تشير النتائج التي توصلنا إليها إلى أن الإصابة بفيروس الهربس البشري النوع الثامن وتعدد أشكال الجينات للجين المثبط لنمو الخلايا السرطانية يمكن أن يؤثرا على بيولوجيا وتطور الورم اللمفاوي.

الكلمات المفتاحية: –

فايروس الهربس البشري النوع الثامن، سرطان الغدد اللمفاوية، تقنية تفاعل البلمرة المتسلسل، الجين المثبط لنمو الخلايا السرطانية، تعدد الاشكال الوراثية، تسلسل الحامض النووي.