

### Inducing of Interleukins-10 and 8 by Epstein Barr Virus in Chronic Lymphocytic Leukemia

*Luma A. Yassir<sup>1</sup>, Amer Alnajjar<sup>2</sup>, Dawood S. Dawood<sup>3</sup>, Alaa Fadhil Alwan<sup>4</sup>*

1 MSc microbiology/ The national center of hematology/Almustansiriya University/ Baghdad/Iraq

2 Prof. of medical virology/ head Dept. of microbiology / Almustansiriya medical college/Baghdad/Iraq

3 Assistant prof. of medical virology/ Dean of medical technical college/Baghdad / Iraq

4 Prof. of clinical hematology/ director of national center of hematology/Baghdad/ Iraq

---

Received: 2/12/2015

Accepted: 14/ 12/ 2015

#### Abstract:

**Background:** Many newer studies reported that Epstein- Barr virus (EBV) has association with chronic lymphocytic leukemia (CLL). The average age of presentation is of patients with CLL is between 65 and 70 years with male to female ratio is 2:1. Notably, several studies have reported that expression of Epstein Barr encoding RNA (EBERs) is associated with progressive or accelerated clinical courses. This type of RNA increases the level of IL-8 and IL-10 in serum of newly diagnosis of CLL patients.

**Objectives:** the aims of study were to determine the interleukins 10 and 8 level in newly diagnosed CLL patients and determine the incidence of EBV infection in patient of CLL.

**Materials and Methods:** A prospective study conducted at department of clinical hematology in the national center of hematology in Baghdad, Iraq from January 2013 to January 2014. It included thirty samples of formalin-fixed, paraffin-embedded tissue of bone marrow aspirates samples and blood from newly diagnosis B-CLL. They were diagnosed with CLL according to Binet criteria. The detection of EBV encoded RNAs (EBER1, EBER2) and also detection for the level of Interleukin (8 and 10) in the serum of CLL patients were done by in situ hybridization technique.

**Results:** Histopathological study revealed that all the controls were negative for EBERs and 46.7% were found to be EBERs positive. There was a correlation between positive EBERs and tumors stage and also EBERs and IL-10 and with IL-8.

**Conclusion:** The highest incidence of CLL occurs in the age group 40-80 years old and males are more liable than females (male/female: 70/30). In situ hybridization technique is successful method to detect of EBV and positive EBERs. IL-8 is highly significant in CLL patients and correlates with EBERs and LMP1. There is a correlation between IL-10 EBERs and LMP1 in CLL patients.

**Keywords:** Epstein-Barr virus, CLL, EBERs, IL-8, IL-10

## Introduction

Chronic lymphocytic leukemia (CLL) results from neoplastic proliferation of a mature B by the accumulation of non-dividing Small lymphocytes. It is characterized by a persistent lymphocytosis of more than  $10 \times 10^9$ /liter and lymphoid intrusion of the bone marrow of at least 40% <sup>(1,2)</sup>

With the use of immunological markers, it is likely to establish the diagnosis of CLL by B cell clonality, even with lymphocyte counts of less than  $5 \times 10^9$ /liter. CLL is documented as a disease entity in the WHO organization and as the leukemic counterpart of small lymphocytic lymphoma and CLL the most common type of adult leukemia in the United States and Western Europe. <sup>(3)</sup>

The mutation status of the immunoglobulin heavy chain variable region (Ig VH) genes can differentiate between these two groups: CLL patients with unmutated immunoglobulin high variable (Ig VH) genes have a negative prognosis with rapid progression of the disease. <sup>(3)</sup>

Epstein- Barr virus (EBV) infection is only occasionally detected in CLL by conventional diagnostic approaches. This is consistent with in vitro results suggesting that CLL cells do not

regularly become activated or immortalized after exposure to EBV, although this can be achieved after cytokine activation. <sup>(4)</sup>

Epstein-Barr virus is the first virus described to be linked with the human pathogenesis of tumor. The topographical distribution of Burkitt lymphoma were related to areas endemic with falciparum malaria is supposed to cause chronic excitement or suppression of the immune system, making children more susceptible to the oncogenicity of EBV. <sup>(5,6)</sup>

In 1968, EBV was recognized to be the etiological factor of infectious mononucleosis. At the same time, EBV was reported to alter infected B cells to uncontrolled proliferation. <sup>(7)</sup> A large study in 2009 determined around one in every ten stomach cancers contained EBV. Studies are ongoing to decode what role the virus is playing in this type of cancer and how it weaves together with other risk factors like nutrition, genetics, and infection of *H. pylori*. <sup>(8)</sup>

Initiation of EBV lytic program occurs in memory B cells recirculating during the lymphoid tissue related with the oropharyngeal mucosa. Host

immunosuppression may also generate viral reactivation in lately infected B cells, which leads to creative infection. Nevertheless, the original mechanism of viral reactivation *in vivo* is not clearly understood.<sup>(9)</sup>

EBV alters B-lymphocyte growth, causing permanent growth transformation by regulated expression of multiple viral genes. These genes comprise three integral membrane proteins, latent membrane proteins 1, 2A, and 2B (LMP), 6 EBV nuclear antigens (EBNA1, 2, 3A, 3B, 3C, and EBNA-LP), then two small, non-coding nuclear RNAs (EBERs). The gene linked products relate with or present homology to various antiapoptotic molecules, cytokines, and signal transducers, endorsing EBV infection, immortalization, and alteration.<sup>(10)</sup>

There are two types of EBV viruses , EBV-1 and EBV-2, which vary depend on the EBV nuclear antigen (EBNA) genes.<sup>(11)</sup> EBV-1 is more common in most populations and is more competent in transforming B cells *in vitro*. EBV-2 is mainly found in parts of Africa and is related endemic Burkitt lymphoma.<sup>(12)</sup>

## Material and Methods:

This study was designed as a prospective (case-control) study conducted on the following main groups during the period from January 2013 to January 2014. Thirty formalin-fixed, paraffin embedded tissue blocks were obtained from Bone marrow biopsies and blood samples of B-CLL patients.

The age of the patients ranged between 40-80 years, and the samples were collected directly from patients in Baghdad Teaching Hospital, from the National Center of Hematology and Twenty Bone marrow biopsies and blood sera of 20 patients who have hematological problem other than CLL as control.

We use In situ hybridization to detected EBERs in Bone marrow samples In situ hybridization detection kit from abcam lot-S01\_M61, REF \_ WB. 005.50: Hybridization / detection system for EBV was purchased from ZytoFast /Germany Cat. Numbers (T-1070-40) and ELISA kit for detection Human IL-10 and IL-8 by (abcam46059 lot:GR162207- 4) and (abcam46032 the lot: GR151489-6) .

## Results:

The distribution of age in the studied group ranged between 40 - 80 years with a mean of (60.4) year (table 4.1). Males constituted 70% of cases and female 30%. The ages of control groups ranged between 42 - 70 years with a mean of (55.8) year. Males constituted 76.2% of control groups and female constituted 23.8%. (Table 1).

As shown in table (2), all controls were negative for EBERs. Although the median score and intensity for EBERs was negative for cases group, the mean rank for EBERs score, intensity and composite score (a score resulting from multiplying the score by intensity) was

significantly higher among cases compared to controls, figure (1),(2) ,(3) and Figure( 4 ) show the result of In situ hybridization. As shown in table (3), the median IL8 was significantly higher in CLL cases group (33.1 pg/ml) compared to control group (22.1 pg/ml), also in figure (5). A similar pattern was applicable to IL10. The median IL10 was also significantly higher in CLL cases group (29.1 pg/ml) compared to control group (0 pg/ml), figure (6) . As shown in table (4), the positive test for EBERs had no obvious or statistically significant association with IL8 concentration. IL10 concentration also failed to show any noticeable linear correlation with similarly measured IL8 concentration.

**Table (1): CLL patients distribution according the age and gender**

		CLL		Control	
		No	%	No	%
Age (years)	<50	5	16.7	4	19.0
	50---	7	23.3	9	42.9
	60---	14	46.7	7	33.3
	=>70	4	13.3	1	4.8
	Mean±SD(Range )	60.4±9.2 (40-80)		55.8±8.1 (42-70)	
	P value	0.392			
Gender	Male	21	70.0	16	76.2
	Female	9	30.0	5	23.8
	P value	0.626			

\*Significant difference between proportions using Pearson Chi-square test at 0.05 level

**Table 2 : The case-control difference in median score and intensity and composite score for EBERs viral marker.**

	Study group				P
	Controls		Cases (CLL)		
	N	%	N	%	
<hr/>					
1. EBERs-score					
Negative	20	100.0	16	53.3	
+	0	0.0	11	36.7	
++	0	0.0	3	10.0	
Total	20	100.0	30	100.0	
Range	(Negative to Negative)		(Negative to ++)		<0.001
Median	Negative		Negative		
Inter-quartile range	(Negative to Negative)		(Negative to +)		
Mean rank	18.5		30.2		
2. EBERs-intensity					<0.001
Negative	20	100.0	16	53.3	
Weak	0	0.0	8	26.7	
Moderate	0	0.0	5	16.7	
High	0	0.0	1	3.3	
Total	20	100.0	30	100.0	
Range	(Negative to Negative)		(Negative to High)		
Median	Negative		Negative		
Inter-quartile range	(Negative to Negative)		(Negative to weak)		
Mean rank	18.5		30.2		
EBERs-composite score (score x					
3. intensity)					<0.001
Range	(Negative to Negative)		(Negative to 6)		
Median	Negative		Negative		
Inter-quartile range	(Negative to Negative)		(Negative to 1)		
Mean rank	18.5		30.2		

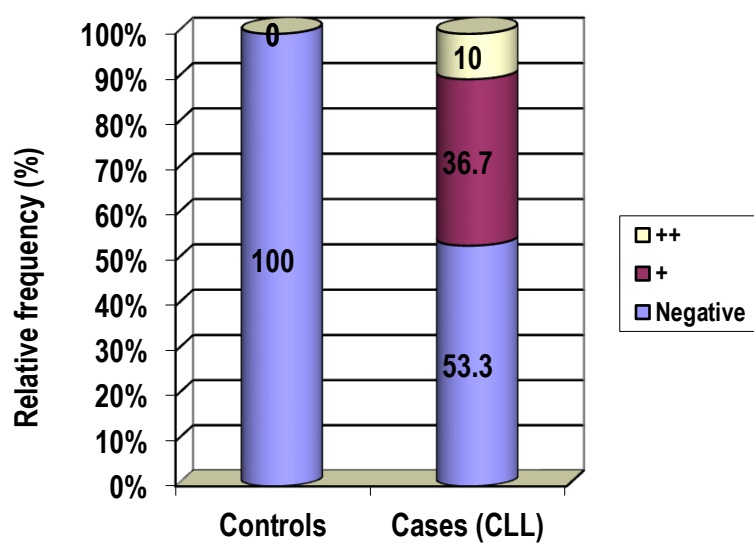


Figure 1: Component bar chart showing the case-control difference in EBERs-score.

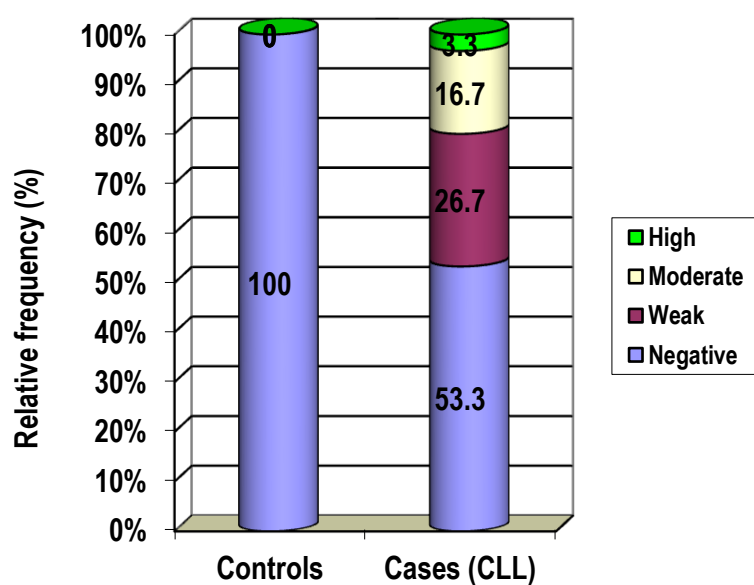
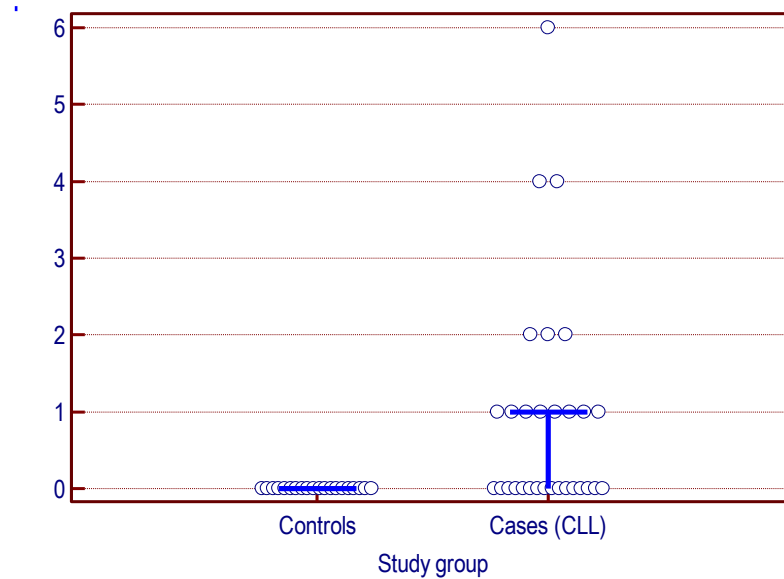
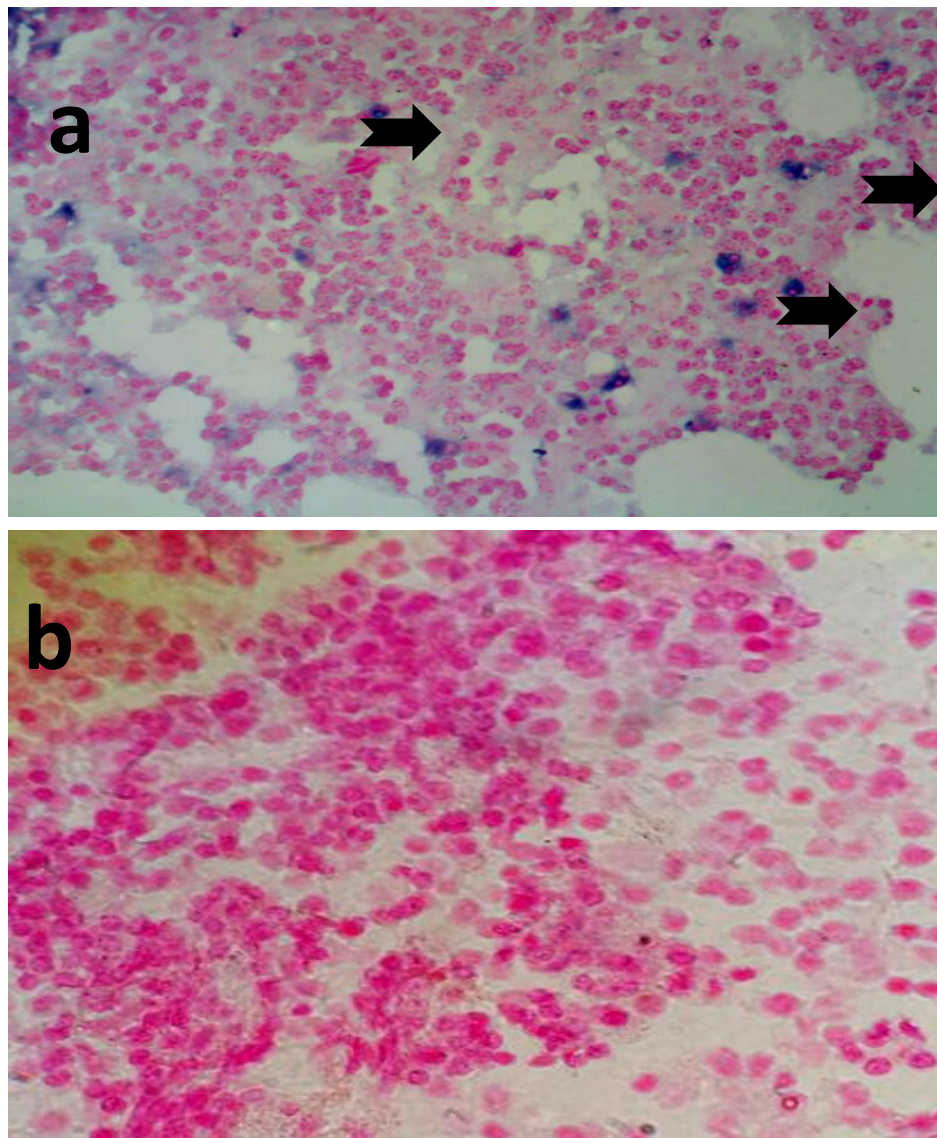


Figure 2: Component bar chart showing the case-control difference in EBERs-intensity.



**Figure 3 : Dot diagram with error bars showing the median (with its inter-quartile range) EBERSs in cases with CLL compared to controls.**

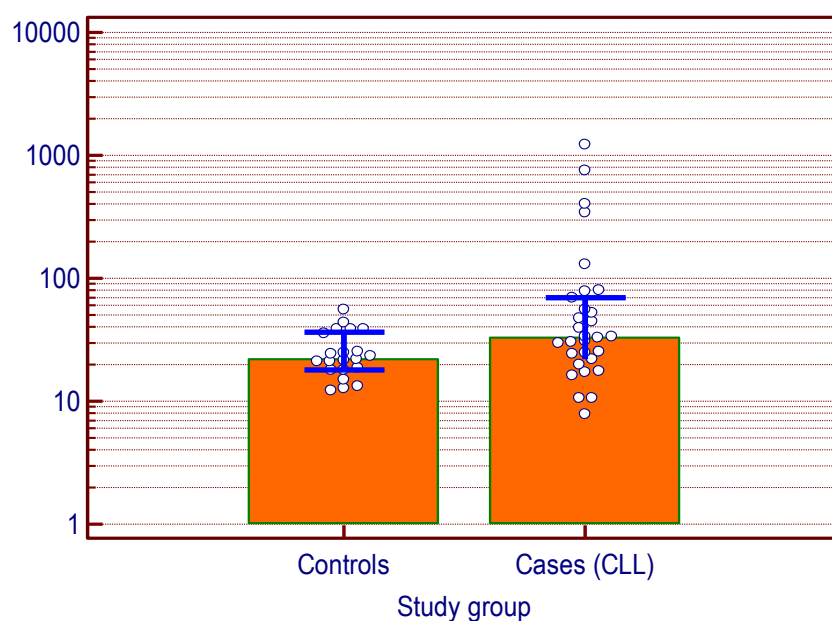


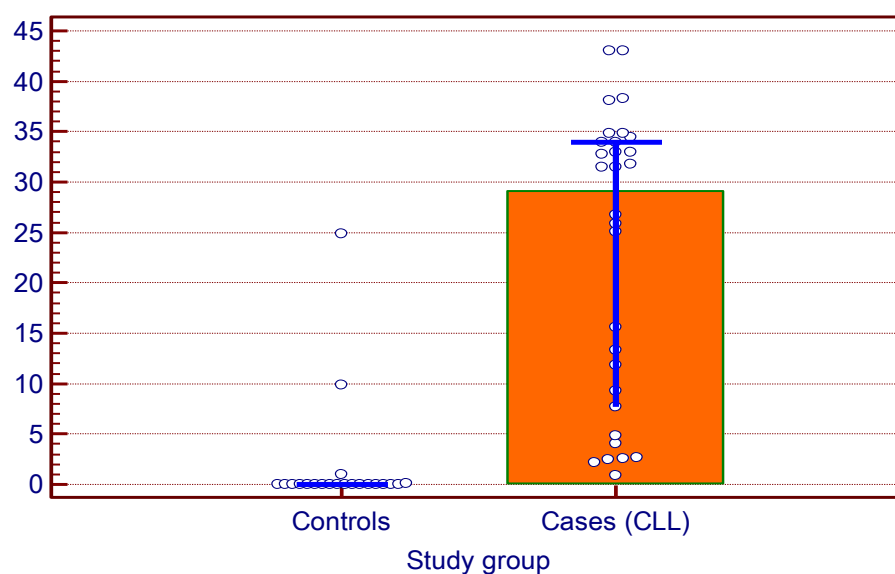
**Figure 4** : Representative expression of EBV-encoded small nuclear early region (EBERs) in Bone Marrow of CLL patients d . **a** : **positive result show the** Nuclear localization of the EBERs in neoplastic cells by ISH . **b** : **Negative result.**



**Table 3 : The case-control difference in median interleukin concentration.**

	Study group		P
	Controls	Cases (CLL)	
IL8 (pg/ml)			0.03
Range	(12.2 - 55.3)	(7.8 - 1215)	
Median	22.1	33.1	
Inter-quartile range	(17.9 - 35.4)	(21.9 - 70.1)	
N	41	30	
Mean rank	20.6	29.8	
IL10 (pg/ml)			<0.001
Range	(0 - 24.9)	(0.9 - 43)	
Median	0	29.1	
Inter-quartile range	(0 - 0)	(7.7 - 33.9)	
N	41	30	
Mean rank	12	35.8	

**Figure 5 : Dot diagram with error bars showing the median (with its inter-quartile range) IL8 (pg/ml) in cases with CLL compared to controls. (Logarithmic scale was used)**



**Figure 6 : Dot diagram with error bars showing the median (with its inter-quartile range) IL10 (pg/ml) in cases with CLL compared to controls.**

**Table 4 : The median IL8 concentration (pg/ml) by selected explanatory variables among cases with CLL.**

			IL8 Interquartile range	N	Mean Rank	P
	Range	Median				
Age group (years)						0.6[NS]
<60	(10.6 to 79.4)	31.9	(22.2 to 61.3)	12	14.5	
60+	(7.8 to 1215)	36	(21.9 to 130.3)	18	16.2	
Gender						0.5[NS]
Female	(7.8 to 1215)	55.6	(20 to 401.5)	9	17.2	
Male	(10.6 to 346.2)	32.8	(24.3 to 46.9)	21	14.8	
Positive EBERs						0.1[NS]
Negative	(10.6 to 1215)	43.1	(25.3 to 104.9)	16	18	
Positive	(7.8 to 401.5)	30.4	(17.7 to 44.4)	14	12.7	

**Table 5 : The median IL10 concentration (pg/ml) by selected explanatory variables among cases with CLL.**

			IL10 Interquartile		Mean	
	Range	Median	range	N	Rank	P
Age group (years)						0.18[NS]
<60	(0.9 to 38.3)	20.35	(6.25 to 29.85)	12	12.9	
60+	(2.5 to 43)	32.3	(9.3 to 34.8)	18	17.3	
Positive EBERs						0.63[NS]
Negative	(0.9 to 43)	20.75	(8.5 to 33.9)	16	14.8	
Positive	(2.2 to 43)	32.3	(4 to 33.9)	14	16.3	

**Discussion :**

Epstein- Barr virus has strong association with variety in B-cell tumors including Burkitt's lymphoma, Hodgkin lymphoma, human immunodeficiency virus, post transplantation lymphoma disorder and chronic lymphocytic leukemia. Many studies reported that CLL patient had evidence of EBV infection by In situ hybridization for EBERs and detection of EBV-encoded EBER transcripts is considered the gold standard for localizing latent EBV in tissue samples, as EBER transcripts are universally expressed in all EBV associated tumors.<sup>(13,14)</sup>

The presence of EBERs has been shown to correlate with progressive or accelerated clinical course including transformation to Richter's large cell lymphoma.<sup>(15,16)</sup>

However EBERs is also found in quiescent EBV latency where no protein is produced and that may be a suboptimal marker for proliferation or transformation capability.

In this study all controls showed negative result EBERs but in patients the result show that 14 (46%) out of 30 CLL patients were positive with EBERs.

Results obtained are nearly compatible to previous study who reported that (38%) of CLL patients had evidence of EBV infection proved by EBERs positively in tumor cells<sup>(16)</sup>.

Result demonstrated in this study were in accordance with 16) Tsimberidou *et al* who stated that 12 out of 32 CLL patients has appositive result.<sup>(16)</sup>

On the other hand another study found that 8 of 75 (10.7%) cases showed EBERs expression restricted to 5–10% of tumor cells.<sup>(17)</sup>

This controversy in the above results may be related to that ISH process depends on the RNA staining and the concentration of RNA in the cell .this method affected by many factors, including the RNA present in the cell and concentration of RNA. This technique is very sensitive. Interleukins-8 and it's receptor increased in cancer cells, infiltrating neutrophils, endothelial cells, and tumor-associated macrophages<sup>(18)</sup>

There is no evidence of IL-8 production by normal B cells but many studies showed that the natural cellular source of IL-8 production have been described to be monocyte/macrophages, T cells, large granular lymphocytes, fibroblasts, endothelial cells, mesothelial cells, keratinocytes, neutrophils ,hepatocytes and chondrocytes.<sup>(19,20,21)</sup>

A study which has been done by Celle et al showed that elevated IL-8 levels may be founds in the serum of untreated B cell patient which may be release by B cells and superannuated of purified B-CLL cells contain IL8 released chemotactic activity for neutrophils.<sup>(22)</sup>

In our study we found that IL-8 was significantly higher in CLL cases group the compared with control group. This result is compatible with other study published<sup>(23,24)</sup> which found that plasma IL-8 level enhances in CLL patients. The same result was studied by Yoshizaki et al who found that IL-8 increased in CLL patients.<sup>(25)</sup> On the other hand another study found that serum IL-8 level was not increased in significant level in patient with CLL when she compared with healthy control.<sup>(26)</sup>

The association of EBERs and IL-8 did not reach the level of statistical significance. This result is not compatible with study done by William et al who found that the stages were associated with significantly higher plasma IL-8 levels ( $P < 0.0001$ ) but There were no significant difference between IL-8 production and gender.<sup>(27)</sup> While another study found compatible result with this study where IL8 level in CLL patients not correlated with CLL stages.<sup>(24)</sup>

Notably, serum IL-10 levels are increased in CLL patients and correlate with adverse disease features and short survival.<sup>(28,29)</sup>

Results obtained in this study revealed that the IL-10 was significantly higher in CLL cases group median rang (29.1 pg.

/ml) compared to control group (0 pg/ml) and the mean rank 35.8 with  $p < 0.001$  figure (6)

This result is compatible with other study<sup>(30)</sup>, Which found that IL-10 levels were higher in CLL patients (median, 5.04 pg/mL; range, undetectable to 74 pg/mL) than in normal volunteers (median, undetectable; range, undetectable to 13.68 pg/mL) ( $P < .00001$ ), and another study found that IL-10 levels increased in CLL patient of Iraq and significantly than control group  $P < 0.05$  same study was studied by [31] who found that Serum levels IL-10 in 20 CLL patient severely dropped in untreated group ( $27 \pm 11.47$  and  $0.65 \pm 0.23$  pg/mL respectively) and differed significantly healthy in 20 control group ( $1715.66 \pm 1014$  pg/mL respectively)<sup>(26)</sup> and David et al who found that Serum IL-10 levels were also significantly elevated in CLL patients.<sup>(18)</sup>

Other explanations for the increase in the level of IL-10 in patient with CLL was demonstrated in other researches which found that IL-10 is increased in production by culture of CLL and that serum IL-10 levels were elevated in five of the eleven B-CLL patients. These findings suggest that IL-10 acts as an autocrine growth factor for B-CLL cells and cytokine-based therapy might be a

new approach for the treatment of B-CLL.<sup>(33,34)</sup>

IL-10 was derived from EBV infected tumor cells and demonstrated in serum of CLL and Hodgkin lymphoma patients.<sup>(30,34)</sup> Several studies have reported an association between EBERs, LMP1 and IL10 stimulation. EBV infection enhanced production of viral IL-10 and may also contribute to a local immune suppression by production of hydrophobic peptides derived from the first transmembrane domain of LMP-1<sup>(35)</sup> In contrast EBERs induce the transcription of various cytokines depending on cell type, such as interleukin-10 (IL-10).

**Conclusion:** The highest incidence of CLL occurs in the age group 40-80 years old and males are more liable than females (male/female: 70/30). In situ hybridization technique is successful method to detect of EBV and positive EBERs. IL-8 is highly significant in CLL patients and correlates with EBERs and LMP1. There is a correlation between IL-10 EBERs and LMP1 in CLL patients.

## References:

1. Estella M. Claire D. Chronic lymphocytic leukemia, The Medicine Publishing Company Ltd , (2004) . 125(2):P15-38.
2. Garcia-Muñoz R. & Galiacho V. R . Immunological aspects in chronic lymphocytic leukemia (CLL) development. Ann Hematology .(2012) . 91:981–996.
3. Byrd J.C. , Stilgenbauer S. , and Flinn I.W. Chronic lymphocytic leukemia. Hematology. Am. Soc. Hematology. (2004) Educ.V,11(3): p. 163-183.
4. Mao Z. , Quintanilla-Martinez L. , Raffeld M. , Richter M. , et al. IgVH mutational status and clonality analysis of Richter's transformation: diffuse large B-cell lymphoma and Hodgkin lymphoma in association with B-cell chronic lymphocytic leukemia (B-CLL) represent 2 different pathways of disease evolution.(2007). Am J Surg Pathol. 31(10): p. 1605-14.
5. Hjalgrim H , and Engels EA. Infectious aetiology of Hodgkin and non-Hodgkin lymphomas: a review of the epidemiological evidence. Journal of internal medicine. (2008); 264(6): p. 537-48.
6. Burkitt D. Etiology of Burkitt's lymphoma--an alternative hypothesis to a vectored virus. Journal of the National Cancer Institute. (1969) ;42(1): p. 19-28.
7. Diehl V. , Henle G. , Henle W. , and Kohn G. Demonstration of a herpes group virus in cultures of peripheral leukocytes from patients with infectious mononucleosis. Journal of virology. (1968); 2(7): p. 663-9.
8. Murphy G. , Pfeiffer R. , Camargo M.C. , and Rabkin C.S. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. Gastroenterology. (2009);137(3): p. 824-33.
9. Ayumi K., Masatoshi F., Tohru K. et al . Inhibiting Cellular DNA Replication Cyclin-Dependent Kinase Activity while Virus Occurs with High S-Phase Cells Latently Infected with Epstein-Barr: Journal of virology .(2003) ;77(2):851.
10. Aylın F. K., Özyar EN. S., Ayfie A. A. University, Epstein-Barr virus genes and nasopharyngeal cancer. Turkish Journal of Cancer. (2006). Volume: 36, No.3.
11. Bortolin M T , Pratesi C , Dolcetti R et al., Clinical value of Epstein–Barr virus DNA levels in peripheral, blood samples of Italian patients with undifferentiated carcinoma of nasopharyngeal cancer type. Letters 233. (2006) ; 247–254. .
12. Young LS , Yao QY , Rooney CM , Sculley TB , et al. New type B isolates of

Epstein-Barr virus from Burkitt's lymphoma and from normal individuals in endemic areas. *The Journal of general virology*. (1987); 68(Pt 11): p. 2853-62.

13.Tsimberidou AM , Keating MJ , Bueso-Ramos CE , and Kurzrock R Epstein-Barr virus in patients with chronic lymphocytic leukemia: a pilot study. *Leuk Lymphoma*. (2006); 47(5): p. 827-36.

14.Neparidze N , and Jill M.D. Malignancies Associated With Epstein-BarrVirus: Pathobiology, Clinical Features, and Evolving Treatments , *Clinical Advances in Hematology & Oncology*.(2014); 12(6).

15.Ansell S.M., Li C.Y., Lloyd R.V., Phylly RL. Epstein-Barr virus infection in Richter's transformation. *Am J Hematol*. (1999) ;60(2):99-104.

16.Tsimberidou AM , Keating MJ , Bueso-Ramos CE , and Kurzrock R. Epstein-Barr virus in patients with chronic lymphocytic leukemia: a pilot study. *Leuk Lymphoma*. (2006) ; 47(5): p. 827-36.

17.Cohen J.I. Epstein-barr virus vaccines, *Clinical & Translational Immunology* ; doi (2014):10.27.

18.David J. DiLillo, J. Brice Weinberg, Ayumi Yoshizaki, Mayuka Horikawa, Jacquelyn M.Bryant, Yohei Iwata, Takashi Matsushita, Karen M. Matta,

Youwei Chen, Guglielmo M. Venturi, Giandomenico Russo, Jon P. Gockerman, Joseph O. Moore, Louis F. Diehl,Alicia D. Volkheimer, Daphne R. Friedman, Mark C. Lanasa, Russell P. Hall, and Thomas F. Tedder,Chronic Lymphocytic Leukemia and Regulatory B Cells ShareIL-10-Competence and Immunosuppressive Function , *Leukemia*. (2013) ; 27(1): 170–182

19.Smyth M.J., Zachariae C.O.C., Norihisa Y., Ortaldo J.R., Hishinu A., Matsushima K . IL-8 gene expression and production in human peripheral blood lymphocyte subsets. *J Immunology*.(1991); 146:3815.

20.Jonjic N, Peri G, Bernasconi S, Sciacca FL, Colotta F, Pelicci PG, Lanfranccone L, Mantovani A. Expression of adhesion molecules and chemotactic cytokines in cultured human mesothelial cells. *J Exp Med* . (1992). 176:1165.

21.Strieter RM, Chensue SW, Standiford TJ, Basha MA, Showell HJ, Kunkel SL .Disparate gene expression of chemotactic cytokines by human mononuclear phagocytes. *Biochem Biophys Res Commun* (1992) .1662386.

22.Celle P.F. , Carbone A. , Marchis D., Zhou D, Zupo, Massimo Pini et al .Cytokine Gene Expression in B-Cell Chronic Lymphocytic Leukemia

Evidence of Constitutive Interleukin-8 (IL-8) mRNA Expression and Secretion of Biologically Active IL-8 Protein, Blood.(1994); 84(1) : p 220-228.

23.Wierda, G, William Marcella M. Johnson,2 Kim-Anh Do, et al., Plasma interleukin 8 level predicts for survival in chronic lymphocytic leukaemia , British Journal of Haematology.(2003) ; 120, 452–456.

24.Kara I.O., Sahin B., Gunesacar R .Expression of soluble CD27 and interleukins-8 and -10 in B-cell chronic lymphocytic leukemia. Correlation with disease stage and prognosis.(2007); 24(1) : pp 29-40.

25.Yoshizaki T, Horikawa T, Qing-chun R, Wakisaka N et al. Induction of Interleukin-8 by Epstein-Barr Virus Latent Membrane Protein-1 and Its Correlation to Angiogenesis in Nasopharyngeal Carcinoma, Clinical Cancer Research. (2001);Vol. 7, 1946–1951.

26.Ahmed N. Sh., Saad M. Nada, Hadi Y. A. , Ibraheem A. N., Jaafar M.R. Expression of Cytokine of Chronic Lymphocyte Leukemia from Baghdad / Iraq Population, Iraqi Journal of Cancer and Medical GeneticsVolume. .(2014);7 (2).

27.William G. Wierda,1 Marcella M. Johnson,2 Kim-Anh Do,et al. Plasma interleukin 8 level predicts for survival in chronic lymphocytic leukaemia , British Journal of Haematology. (2003) ; 120, 452–456.

28.Dolcetti R. , and Masucci M.G. Epstein-Barr virus: induction and control of cell transformation. J Cell Physiol. (2003). 196(2): p. 207-218.

29.Vockerodt M , Morgan SL , Kuo M , Wei W , et al. The Epstein-Barr virus oncoprotein, latent membrane protein-1, reprograms germinal centre B cells towards a Hodgkin's Reed-Sternberg-like phenotype. J Pathol. (2008); 216(1): p. 83-92

30.Fayad L., Keating M. J., Reuben M. J. et al. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia:correlation with phenotypic characteristics and outcome .by The American Society of Hematology. Blood. (2001); 97 (1).

31.Al-Dabagh M. A. and Al-Mayah K. Sh. Evaluation of immune response in patients with chronic lymphocytic leukemia II: Humoral immune response. Iraqi journal of science , (2011) ;Vol .52,No.3, ,P.388-393.

32.Kitabayashi A., Kirokawa M., Miura A.B . The role of interleukin-10 (IL-10) in chronic B-lymphocytic leukemia: IL-



10 prevents leukemic cells from apoptotic cell death. *Int J Hematology*. (1995); 62:99-106.

33. Tangye S.G., Weston K.M., Raison R.L. Interleukin- 10 inhibits the in vitro proliferation of human activated leukemic CD51 B-cells. *Leuk Lymphoma*. (1998); 31:121-130.

34. Blay J.Y., Voorzanger N., Favrot M., Burdin N., Rousset F., Banchereau J. Presence of Epstein-Barr virus viral interleukin 10 in the serum of patients with non-human-immunodeficiency-virus related diffuse large-cell non-Hodgkin's lymphomas. *Blood*. (1995). 86:4702-4704.

34. Bandobashi K. , Liu A. , Mi Nagy N. et al .EBV Infection Induces

Expression of the Transcription Factors ATF-2/c-Jun in B Lymphocytes but not in B-CLL Cells *Virus Genes* ,(2005); 30:3, 323–330.

35. Naji A. S. Outcome of 49 Iraqi adult patients with Chronic Lymphocytic Leukemia treated with oral alkylating agent:, *J Fac Med Baghdad*. (2012); 54 (2).

---

### Correspondance to

Luma Amer MSc.

Dept. of molecular genetic

The national center of hematology

Almustansiriyah university

## تحفيز الانترلوكين-10 و 8 بواسطة فيروس ابشتاين بار في سرطان الدم الليمفاوي المزمن

لمى عامر ياسر 1، عامر النجار 2 ، داود سلمان داود 3 ، علاء فاضل علوان 4

- 1 ماجستير علم الأحياء المجهرية / المركز الوطني للأمراض الدم / الجامعة المستنصرية / بغداد / العراق
- 2 أستاذ علم الفيروسات الطبية / رئيس قسم الأحياء المجهرية / كلية طب المستنصرية / بغداد / العراق
- 3 أستاذ مساعد. علم الفيروسات الطبية / عميد كلية التقنية الطبية / بغداد / العراق
- 4 أستاذ أمراض الدم السريرية / مدير المركز الوطني للأمراض الدم / بغداد / العراق

### الملخص:

**الخلفية:** اثبتت الدراسات ان الابشتاين بار فايروس له علاقة ويعتبر احد المسببات لمرضى سرطان الدم اللمفاوي المزمن في هذه الدراسة كان معدل عمر المرضى يتراوح بين (65-70) عام وكانت نسبة الذكور الى الاناث تتراوح بين 1/2 % في مرضى سرطان الدم اللمفاوي المزمن وكما اثبتت هذه الدراسة ان EBERS يزيد من افراز IL-10 و IL-8 في مصول المرضى .

**المواد والطرق:** دراسة مستقبلية أجريت في قسم علم أمراض الدم السريري في المركز الوطني للأمراض الدم في بغداد، العراق خلال الفترة من يناير 2013 إلى يناير 2014. وشملت ثلاثين عينات من والأنسجة الثابتة بالفورمالين جزءا لا يتجزأ من البارافين من عينات نخاع العظام والدم من حديثي التشخيص ب سرطان الدم اللمفاوي المزمن .. وقد أجريت للكشف عن الرنا EBV المشفرة (EBER1، EBER2)، وكذلك الكشف عن مستوى انتروكين (8 و 10) في مصل مرضى سرطان الدم اللمفاوي المزمن بتقنية التهجين الموقعي.

**النتائج :** كشفت الدراسة النسيجية أن جميع الضوابط كانت سلبية EBERS ووجد 46.7% إيجابية من EBERS في سرطان الدم اللمفاوي المزمن . كان هناك ارتباط بين EBERS الإيجابية ومرحلة الأورام وأيضا EBERS و IL-10 و مع IL-8.

**الاستنتاج:** يحدث أعلى معدل لانتشار سرطان الدم اللمفاوي المزمن في الفئة العمرية 40-80 سنة والذكور أكثر عرضة من الإناث (ذكور / إناث: 30/70) . تقنية التهجين الموقعي هي طريقة ناجحة للكشف عن EBV و EBERS الإيجابية. IL-8 مهم للغاية في المرضى الذين يعانون من سرطان الدم اللمفاوي المزمن ويرتبط EBERS و LMP1. هناك علاقة بين IL-10 EBERS و LMP1 في مرضى سرطان الدم اللمفاوي المزمن.

**كلمات البحث:** فيروس ابشتاين بار، سرطان الدم اللمفاوي المزمن ، IL-8، IL-10