DocID: https://connectjournals.com/03896.2020.20.6147

Biochem. Cell. Arch. Vol. 20, No. 2, pp. 6147-6149, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

MOLECULAR INVESTIGATION OF EPSTEIN-BARR VIRUS IN IRAQI PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

Noor Tarek Naeem* and Luma Hasan Alwan

Department of Biology, College of Science for Women, University of Baghdad, Iraq.
*e-mail : noortarik8@gmail.com

(Received 2 June 2020, Revised 14 August 2020, Accepted 19 August 2020)

ABSTRACT : Chronic lymphocytic leukemia (CLL) is one type of leukemia that arises from lymphocytes progenitor cell in the bone marrow, it affects individuals over the age of (50) years in both genders, in Iraq leukemia affected 1532 (847 males and 683 females) according to the latest announced statistics of the Iraqi Cancer registry center at 2012. Chronic lymphocytic leukemia may be occurred due to several genetic causes such as chromosomal aberrations andgene mutations, or exposure to carcinogenesis and mutagens such as (radiation, chemicals and oncogenic viruses), the most famous virus Epstein-Barr virus (EBV), which is a γ-herpes virus, that infects more than 90% of individuals people, it is infection mostly is latent infection and the EBV remains latent in memory B-cells. The aim of this study was to determine whether EBV-DNA load in the peripheral blood with CLL patients by using Real time PCR technique. Epstein-Barr virus genome had been shown that 11 (18%) from 61 patients have the virus genome and the number of copies ranged from (1×10^8-2×10^{11}).

Key words : Epstein-Bar Virus (EBV), chroniclymphocytic Leukemia (CLL), Rt PCR.

INTRODUCTION

Epstein-Barr virus (EBV), human herpes virus 4 (HHV-4), which comprises of double-stranded DNA viruses, poised of a DNA core walled by a nucleocapsid and a tegument, with comparatively large genomes (100–200) genes (Rubicz *et al*, 2013). EBV was the first human tumor virus recognized (Baer *et al*, 1984).

Epstein-Barr virus transferred by saliva, contaminating B-lymphocytes and epithelial cells of the oropharynx (Burnham, 2009). Epstein-Barr virus is classified into two subtypes based on sequence variations in genes, (EBV-encoded small RNA: EBERs), six nuclear proteins (EBNA1, EBNA 2, EBNA 3A, EBNA 3B, EBNA 3C and EBNA-LP) and three membrane proteins (LMP-1, 2A and 2B) (Houldcroft and Kellam, 2011). Epstein-Barr virus infection typically occurs through infancy or early childhood with outmedical symptoms and is related with a higher risk of infective mononucleosis (Macsween and Johannessen, 2014). Epstein-Barr virus infection has also been related with some malignant situations including Burkett lymphoma, nasopharyngeal carcinoma, some gastric cancers and Hodgkin lymphoma (Adam *et al*, 2011; Piccaluga *et al*, 2011; Alibek *et al*, 2013; Ali *et al*, 2018). Epstein-Barr virus infects resting (naive) B cells, epithelial cells and memory B cells and replication in epithelial cells of the oropharynx (Shannon-Lowe *et al*, 2011). Chronic lymphocytic leukemia is a malignant production of small clonal B-lymphoid cell (Hussaini *et al*, 2017). CLL the most predominant leukemia among adults (Furman *et al*, 2014). CLL occurs more frequently in males than females (Ahmed *et al*, 2016). CLL is the most common adult leukemia in the United States and Europe, but is rare in Asia (Taneja and Master, 2019). CLL is characterized by the accumulation of CD5+ CD19+ B cells in the peripheral blood, secondary lymphoid organs and bone marrow of patients (Kocher, 2016).

MATERIALS AND METHODS

The studied peripheral blood samples were collected from 61 (34 mal, 27 female) and 40 control individuals who were distributed to 20 males and 20 females. Patients samples were collected after diagnosis in the National Center of Hematology, AL Mustansiriya University by specialized pathologist based on laboratory investigation, clinical examination and biopsy of bone marrow. The patients subdivided in to two group, pretreatment group which includes 24 patients (16 males, 8 females) and the patients with an old diagnosis 37 (18 male, 19 female) who took a therapeutic dose of (flodaraben) ranging from (3-10) confront the control sample of the healthy individuals who did not show any symptoms was satisfactory. 40 individuals (20 males and 20 females).

Table 1 : The sequences of nitrogen bases for primer and probe of EBV.

The gene	The sequences
Primer EBV W-F	GCAGCCGCCCAGTCTCT,3':5'
Primer EBV W-R	ACAGACAGTGCACAGGAGCCT,3':5'
Probe EBV BamH1	FAM-AAAAGCTGGCGCCCTTGC-TAMRA3':5'

DNA purification

DNA was extracted from the blood according to the instructions of kit extracted from Promega, USA.

Electrophoresis

Real time polymerase chain reaction

The polymerase chain reaction technique was usede to estimate the number of DNA copies. It is possible through this technique and in the sense of the standard curve of the samples known number of copies. Determine the number of copies of DNA in anonymous samples this technique has been effectively applied to the calculation of virus concentrations in applied samples. In this experiment, an EBNA1 gene was used within the EBNA1 plasm to prepare information concentrations used in the preparation of the standard curve, which in turn was used to measure the number of copies of the virus genome for the studied samples.

Primer preparation

The primer and probe supplieded by the company (Macrogen) and preparation the (stock solution) by added (Nuclease-Free Water) for the final focus (100 µM) and the (working solution). It was prepared with a final focus (100 µM) by added (10µl) from (stock solution) to (90µl) Nuclease-Free water.

Stander curves preparation

The plasmid bearing the EBNA1 gene was dissolved, which occupies a piece length (3907) base pair. The supplier of the company (Macrogen) by added (100 ML) from Nuclease-Free Water sultanate concentration 4×10^{-8} Mg/ML, which is equivalent 10^{11} Copy of plasmid/ML. It is known that the weight of one copy of plasmid draw 4.282×10^{-18} a decreasing fear chain was prepared number of copies 10^9, 10^8, 10^7, 10^6, 10^5, 10^4 copy/ML. These concentrations were used to conduct a polymerization reaction for standard curve work (Larionov *et al*, 2005).

Table 2 : Materials used in polymerization reaction and quantity.

Materials	Concentration	Volume
Go Taq Probe q PCR Master MIX	2x	10 µl
PrimerF	10 uM	1µL
PrimerR	10 uM	1 µL
Probe	10uM	1µL
Deionized Water		2 µL
DNA		5µL
The final size		20µL

The number of Epstein-Barr (EBV) copies was measured using RT-PCR.

The program was set as shown in Table 3.

Table 3 : RT-PCR Program.

Steps	Time	Temperature	The camera	CT
Primary DNA Mutagenesis	15 min.	95^0C		45 cycle
DNA Mutant	30sec.	$95C^0$	Off	
The correlation of the bulbs	30sec.	$60C^0$	ON	
Elongation	45sec.	$72C^0$	Off	

RESULTS

The number of copies of the virus genome was calculated by the straight-line equation from the graph.

$y = -3.4426x + 51.468$

The molecular study by (real tim Polymerase chain reaction technique) for the detection of the Epstein-Barr virus genome had been shown that 11 (18%) from 61 patients have the virus genomeand the number of copies ranged from (1×10^8-2×10^{11}), these number of copies represents a real or effective infection. But no viral copies have been shown in healthy subjects, while not shown in any sample of healthy samples. In another study by Hong *et al* (2012), where 5000 copies of the virus was considered a threshold.

In a study carried out by Jebbink *et al* (2003) to detect Epstein-Barr virus replication with the technique of polymerase chainreaction, it was found that copies of the virus ranged from 10 to 10 million copies, indicating the activity of the virus as its presence in large numbers is evidence of the spread of the virus (Jebbink *et al*, 2003) and in another study by Gartzonika *et al* (2012) is found (67). People infected with the Epstein-Barr virus of the total (118) renege copy number 5.2×10^3copy \ML ranged of 2.85×10^2-7.6×10^4 copy\ML in the plasma sample.

The number of patients with high copies of the virus who did not receive a therapeutic dose (5 Males, 3 females) in the rate of (33%) of total (24) Patient, may be associated with poor health of patients and immune weakness and the high number of white blood cells in patients before treatment. It is a characteristic of the underlying diseaseand it appeared (8%) of patients after treatment (2 males, 1 females), who carry high copies of

Table 4 : Represents the values of Ct and the number of copies of the virus genome.

The patients	The number	CT value	Copies number of virus
Males before treatment	5	23.97	1×10^{8}
		13.21	2×10^{11}
		14.35	8×10^{10}
		23.4	2×10^{8}
		14.45	8×10^{10}
Male after treatment	2	19.99	2×10^{9}
		23.97	1×10^{8}
Females before treatment	3	14.18	9×10^{10}
		19.83	2×10^{9}
		18.31	6×10^{9}
Females after treatment	1	23.36	2×10^{8}
Healthy			Negative

the viral genome associated with the white blood cell genome indicating their poor immune status despite drug treatment (Flodaraben), which works on urging Apoptosis, In the leukemia cells which leads to kill her and remove the virus (Feng *et al*, 2017). But when a patient relapses between therapeutic doses leads to a rise number of leukemia cells thus increasing the number of virus copies.

These results are consistent with the results of a study conducted by Visco (2015) in Italy it was found that copies of the virus genome higher in patients with chronic lymphocytic leukemia in the rate of 19% of the healthy.

CONCLUSION

Epstein-Barr virus genome had been shown that 18% of the patients have the virus genome.

REFERENCES

Adam P, Bonzheim I, Fend F and Quintanilla-Martínez L (2011) Epstein-Barr virus-positive diffuse large B-cell lymphomas of the elderly. *Adv. Anatomic Pathol.* **18**(5), 349-355.

Ahmed A M B, Yagoub T E, AlSaid A M, Mohammed J S and Osman S M (2016) Chronic leukemia in patients presenting to radiation and isotopes center–Khartoum during period from 1/2006 to 1/ 2007. *Imperial J. Interdiscip.Res.***2**, 211-218.

Ali S H M, Alajeely A A A, AL-Lebawy N S, Al-Alwany S H M and Abed-Alzuwaid A A (2018) Molecular Tracing of Abundances Of Latent Epstein – Barr Virus Early Repeats in Laryngeal Carcinomatous Tissues From a Group of Iraqi Patients: A Possibility of An Early Event in Laryngeal Carcinogenesis, *Biochem. Cell. Arch*. **18**(1), 421-427.

Alibek K, Kakpenova A and Baiken Y (2013) Role of infectious agents in the carcinogenesis of brain and head and neck cancers. *Infectious Agents and Cancer* **8**(1), 7.

Baer R, Bankier A T, Biggin M D, Deininger P L, Farrell P J and Gibson T J (1984) DNA sequence and expression of the B95-8 Epstein—Barr virus genome. *Nature* **310**(5974), 207-211.

Feng Z, Zheng W, Tang Q, Cheng L, Li H, Ni W and Pan X (2017) Fludarabine inhibits STAT1-mediated up-regulation of caspase-3 expression in dexamethasone-induced osteoblasts apoptosis and slows the progression of steroid-induced avascular necrosis of the femoral head in rats. *Apoptosis* **22**(8), 1001-1012.

Furman R R, Sharman J P, Coutre S E, Cheson B D, Pagel J M and Hillmen P (2014) Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N. Eng. J. Med.* **370**(11), 997-1007.

Gartzonika C, Vrioni G, Priavali E, Pappas G and Levidiotou S (2012) Utility of real-time PCR in the diagnosis of primary Epstein-Barr virus infection. *J. Med. Microb.Diagn.* **1**(118), 2161-0703.

Hong J H, Bae Y J, Sohn J H, Ye B I, Chun J K and Kim H M (2012) Plasma real time-quantitative polymerase chain reaction of epstein-barr virus in immunocompetent patients with hepatitis. *Pediatric Gastroenterology, Hepatology& Nutrition* **15**(1), 38-43.

Houldcroft C J and Kellam P (2015) Host genetics of Epstein–Barr virus infection, latency and disease. *Reviews in Medical Virol.* **25**(2), 71-84.

Hussaini M O, Rehman A, Chavez J C, Pinilla-Ibarz J and Horna P (2017) EBV-positive Richter's syndrome with laboratory features of Burkitt's lymphoma, in Ibrutinib-treated chronic lymphocytic leukemia. *Leukemia & Lymphoma* **58**(7), 1753-1756.

Jebbink J, Bai X, Rogers B B, Dawson D B, Scheuermann R H and Domiati-Saad R (2003) Development of real-time PCR assays for the quantitative detection of Epstein-Barr virus and cytomegalovirus, comparison of TaqMan probes, and molecular beacons. *The J. Molecular Diagnostics* **5**(1), 15-20.

Kocher T, Asslaber D, Zaborsky N, Flenady S, Denk U and Reinthaler P (2016) CD4+ T cells, but not non-classical monocytes, are dispensable for the development of chronic lymphocytic leukemia in the TCL1-tg murine model. *Leukemia* **30**(6), 1409-1413.

Larionov A, Krause A and Miller W (2005) A standard curve based method for relative real time PCR data processing. *BMC Bioinformatics* **6**(1), 62.

Macsween K F and Johannessen I (2014) Epstein-barr virus (EBV): Infectious mononucleosis and other non-malignant EBV-associated diseases. In *Viral infections of humans* (pp. 867-896).Springer, Boston, MA.

Piccaluga P P, Gazzola A, Agostinelli C, Bacci F, Sabattini E and Pileri S A (2011) Pathobiology of Epstein–Barr virus–driven peripheral T-cell lymphomas. In: *Seminars in diagnostic pathology* (Vol. 28, No. 3, pp. 234-244). WB Saunders.

Rubicz R, Yolken R, Drigalenko E, Carless M A, Dyer T D, Bauman L, Melton P E, Kent Jr J W, Harley J B, Curran J E and Johnson M P (2013) A genome-wide integrative genomic study localizes genetic factors influencing antibodies against Epstein-Barr virus nuclear antigen 1 (EBNA-1). *PLoS Genet*. **9**(1), p.e1003147.

Shannon-Lowe C and Rowe M (2011) Epstein-Barr virus infection of polarized epithelial cells via the basolateral surface by memory B cell-mediated transfer infection. *PLoS Pathog*. **7**(5), e1001338.

Taneja A, Rettew A C and Master S R (2019) Cancer, Chronic Lymphocytic Leukemia (CLL). StatPearls Publishing LLC.

Visco C, Falisi E, Young K H, Pascarella M, Perbellini O, Carli G and Novella E (2015) Epstein-Barr virus DNA load in chronic lymphocytic leukemia is an independent predictor of clinical course and survival. *Oncotarget* **6**(21), 18653–18663.