

## RESEARCH ARTICLE

# Association of Human Cytomegalovirus with Hodgkin's Disease and Non-Hodgkin's lymphomas

Hamide Mehravaran<sup>1</sup>, Manoochehr Makvandi<sup>1,2\*</sup>, Alireza Samarbaf Zade<sup>1,2</sup>, Niloofar Neisi<sup>2</sup>, Hadis Kiani<sup>2</sup>, Hashem Radmehr<sup>2</sup>, Toran Shahani<sup>2</sup>, Seyedeh Zeinab Hoseini<sup>2</sup>, Nastaran Ranjbari<sup>3</sup>, Rahil Nahid Samiei<sup>1</sup>

### Abstract

**Background and Objective:** The human cytomegalovirus (HCMV) can persist lifelong as a latent infection and may result in a series of disorders. Associations with both Hodgkin's disease and non-Hodgkin's lymphomas have been reported. Expression of the unique long (UL)138 gene of HCMV is linked with the viral latency phase while that of the immediate-early (IE)1 gene is typical of the viral replication phase in patients. This study conducted to determine the prevalence of CMV latent infection in histological tissue samples from patients with Hodgkin's and Non-Hodgkin's lymphomas. **Material and Methods:** A cross sectional study was carried out with a total of 50 paraffin embedded tissues blocks, including 25 samples for Hodgkin's disease and 25 samples for non-Hodgkin's lymphomas. After RNA extraction and cDNA preparation, detection of IE1 mRNA was conducted by RT-PCR and identification of mRNA UL138 was achieved by nested PCR. **Results:** Among 25 cases of Non-Hodgkin's lymphoma, 5 (20%) were positive for UL 138 and 1 (4%) for both IE1 and UL 138. Among 25 cases of Hodgkin only 1 (4%) was positive for UL 138 and all were negative for IE1. **Conclusion:** A relatively high 20% rate of expression of UL 138 was detected in patients with non-Hodgkin's lymphoma, so that latent CMV infection may play a role in development of the disease.

**Keywords:** Human Cytomegalovirus- nested PCR- Hodgkin disease- Non-Hodgkin lymphoma

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### Introduction

HCMV usually results in asymptomatic primary infection, and may persist lifelong in humans, and reactivate sporadically in phases of immunosuppression. In immunosuppressed patients, e.g., transplant recipients and AIDS patients, cytomegalovirus frequently causes severe diseases (do Carmo et al., 2014). It is estimated about 30-90 % of the Western countries and more than 90 % of the developing countries' population are infected with cytomegalovirus (Crough et al., 2009; Lanzieri., 2014). The virus is transmitted via saliva, urine and breast milk or organ transplantation (Swanson et al., 2013). The incubation period is about 4-8 weeks (Schottstedt et al., 2010). Despite the infection of HCMV is commonly asymptomatic, symptoms similar to those of mononucleosis, namely fever, lymph node swelling, gastritis, esophagitis, and flu-like symptoms, are only rarely observed (Crough et al., 2009; Eskandar et al., 2015). HCMV belongs to genus Betaherpesvirinae of the family herpesviridae, HCMV genome is a linear, double-stranded DNA with 230 kb which encodes 200 gene products (Fasanya et al., 2016; Nakase et al.,

2016; Petrucelli et al., 2012). HCMV is not known as an oncogenic virus, but observations demonstrate that HCMV in the latency phase was correlated with certain malignancy such as gastric cancer and T-cell lymphoma (Ponticelli et al., 2011; Jin et al., 2014; Ballanger et al., 2009). Some observations have exhibited that HCMV may be associated with Hodgkin and Non-Hodgkin's lymphomas (Irsai et al., 2012; Tafvizi et al., 2014; Kadry et al., 2014).

Hodgkin and Non-Hodgkin's lymphomas are two malignant lymph nodes (Habibian et al., 2013). Hodgkin Disease is characterized by a small number of putative malignant mononuclear Hodgkin and multinucleated Reed-Sternberg cells among various cells, such as plasma cells, lymphocytes, eosinophils and neutrophils (Randa et al., 2009). Non-Hodgkin's lymphoma includes some malignancies such as lymphoproliferative disorder, Burkitt's and NK-T lymphoma (Bagirath et al., 2014).

After primary infection, HCMV will be entered in the latency phase, which in this phase its genome will become in the form of episome (Goodrum., 2007). There are some factors that implicate in the latency of HCMV. One of these factors is UL138. It is one of the few mRNA genes

<sup>1</sup>Health Research Institute, Infectious and Tropical Diseases Research Center, <sup>2</sup>Virology Department, School of Medicine, <sup>3</sup>Pathology department, ImamhomeiniHospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. \*For Correspondence: manoochehrmakvandi29@yahoo.com

which express during the latency phase. Protein UL138 encoded by a polycistronic locus spanning UL133-UL138 within the ULb' region, which contains genes involved in regulating latency, viral immune escape and cell tropism (Petrucci et al., 2012 ;Goodrum., 2007; Montag et al., 2011). HCMV replication is regulated by subsets of genes, including immediate early genes (IE1), which has an important role in reactivation of latent state (Harwardt., 2016). The detection of UL138 indicates the latent HCMV infection and the detection of IE1 reveals the reactivation of latent CMV infection. The prevalence of Hodgkin and non-Hodgkin's lymphoma have been reported in Iran (Hashemi-Bahremani., 2007). At present diagnosis of HCMV rely on HCMV IgG and IgM which does not describe the exact status of HCMVfection in in patients. On the other hand the performance of real-time PCR and PP65 tests show only the activation or reactivation of HCMV which are costly and not offerable by all patients. Thus this study was conducted to determine IE1mRNA by RT-PCR as a marker for replication or reactivation of HCMV and UL138 mRNA as a marker for latency of HCMV status. To prevent the outcome of HCMV infection in immunosuppression patients and improve treatment, the application of both tets should be implemented in patients with Hodgkin and non- Hodgkin's lymphoma before treatment and during chemotherapy.

The prevalence of HCMV in patients with Hodgkin and non- Hodgkin's lymphoma have not been evaluated in Iran, thus this study was conducted to determine the prevalence of HCMV in latency and reactivation phases in the patients with Hodgkin and non-Hodgkin's lymphoma.

## Materials and Methods

### Patients and Specimens

The cross sectional study was carried out for the total of 50 paraffin embedded tissues blocks, including 25 (50%) samples for Hodgkin disease and 25 (50%) samples for non-Hodgkin's lymphoma. All blocks of lymph node were collected from the department of pathology of Imam Khomeini and Shafa hospitals of the Ahvaz city during 2001-2011. The diagnosis of Hodgkin and Non-Hodgkin's lymphoma were confirmed by the pathologist.

The proposal of this project was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences.

### Deparaffinization and RNA extraction

Initially sections of 5µm were prepared from each paraffin embedded block, after deparaffinazation of the tissues , RNA extraction were performed using

High pure RNA paraffin kit (Roshe, Germany, cat. no. 3270289) according to the manufacturer's instruction. Then complementary DNA (cDNA) was carried out for each extract.

### Nested PCR for UL 138

The Nested PCR was preformed for the each sample in two rounds, in the first round, 25 µl of PCR mixing reaction containing 7µl of extracting mRNA, 2.5µl PCR buffer 10X (Roche), 0.5 µl dNTP 10mM (Roche), 1U Taq polymerase (Roche), 20 pmol of each primer sequence(table1),distilled water up to 25µl, was subjected to Thermal Cycler (Peglab, Germany) and programmed in one cycle 94°C for 5 min, then 40 cycles; 94°C for 1 min, 52°C for one min, 72°C for 1 min and final extension: 72°C for 10 min. For the second round, 5 µl of PCR product of the first run was used in the PCR mixing reaction with the same component described in the first round, was subjected to Thermal Cycler and programmed; One cycle 94C for 5 min, then 35 cycle ; 94°C for 45s, 60° C for 45s, 72°C for 45s and final extension 72°C for 10 min. The primers and amplification conditions shows in Table 1. The final PCR products were subjected to electrophoresis in 2% agarose gel, stained with safe stain and visualized under ultraviolet transilluminator.

### PCR for IE1

The one step PCR was carried out for detection of IE1. The PCR reaction mixture of 25 µl containing 7µl of cDNA mRNA, 2.5µl PCR buffer 10X(Roche), 0.5 µl dNTP 10mM(Roche), 1 U Taq polymerase (Roche), 20 pmol of each primer sequence (table1), distilled water up to 25 µl, was subjected to Thermal Cycler (Peglab, Germany). The thermal program was followed: 1 cycle ,94°C for 5 min, then 40 cycles :94°C for 1 min, 57°C for 1 min, 72°C for

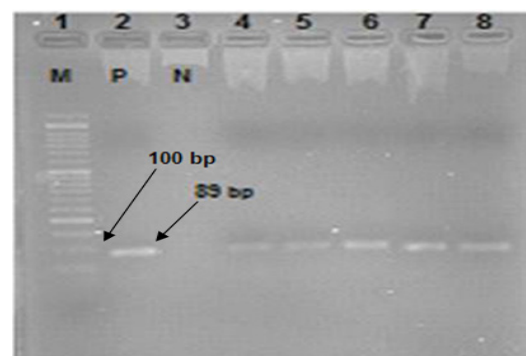


Figure 1. Results of Nested PCR Amplification of UL138 (Positive Band 89 bp) Lane 1:50-bp Size Marker, Lane 2 :Positive Control, Lane 3:Negative Control. Lane 4-8:Non-Hodgkin Samples Positive for UL138.

Table 1. Primers and Amplification Conditions Used for Detection of UL138 and IE1

Name	Primers (5 to 3)		PCR conditions			
	Forward	Reverse	Annealing Temperature C	Annealing	Cycles n	Size bp
UI138	ATGGACGATCTGCCGCTGAA1	TCACGTGTATTCTTGATGAT1	52	60 sec	35	510
IE1	GCTTACCACTGGCAGCACACCT	TACTCCCCGTACAGCTCGCAAC	69	60 sec	40	89
	AGCCTTCCCTAAGACCACCAAT	CATAGCAGCACAGCACCCGACA	57	60 sec	40	260

Table 2. Profile of Patients with Hodgkin and Non-Hodgkin

Category	Male	Female	Total
Hodgkin Lymphoma n = 25			
Mixed Cellularity (MC)	4 (16%)	8 (32%)	12 (48%)
Lymphocyte Predominant (LP)	3 (12%)	2 (8%)	5 (20%)
Nodular Sclerosis (NS)	4 (16%)	4 (16%)	8 (32%)
Non-Hodgkin Lymphoma n= 25			
Diffuse Large Cell Type	4 (16%)	10 (40%)	14 (56%)
Small Lymphocytic Lymphoma		1 (4%)	1 (4%)
High Grade Immunoblastic Lymphoma	1 (4%)		1 (4%)
Mixed Small and Large	2 (8%)		2 (8%)
Malignant T Cell	2 (8%)	2 (8%)	4 (16%)
Malignant B Cell	1 (4%)	1 (4%)	2 (8%)
Diffuse Mixed Large Cell	1 (4%)		1 (4%)

Table 3. Detection of UL138 , IE1 in Subsets of Hodgkin and Non-Hodgkins Lymphoma

Characteristic		Hodgkin (n=25)			Non-Hodgkin (n=25)		
		UL138	IE1	Total	UL138	IE1	Total
Gender	Female	-	-	14	3(12%),2DLBL*1MTC*	1(4%)DLBL*	11
	Male	1(4%)	-	11	2(8%), 1DLBL*1 MTC*	-	14
Age group	<16	-	-	4	-	-	7
	>16	1(4%)	-	21	5(20%)	1(4%)	18

NS\*, Nodular Sclerosis; DLBL\*, Diffuse Large B-cell Lymphoma; MTC\*

1 min and final extension: 72°C for 10 min. The primers and amplification conditions are shown in Table 1. The final PCR products were subjected to electrophoresis in 2% agarose gel, stained with safe stain and visualized under ultraviolet transilluminator.

## Results

Table 2 shows the profile of patients with Hodgkin and Non-Hodgkin lymphoma.

Table 3 shows the results of PCR for UL 138 and IE1 in Hodgkin and Non-Hodgkin groups.

Figure 1 shows the results of nested PCR amplification of UL138 Gene.

## Discussion

Although the association of HCMV and cancer is controversial, but there are evidences revealed that progression of some tumors could be intensified by the regulatory protein named US28 protein encoded by HCMV during the phase of latent infection. HCMV protein, US28 in endogenously infected colorectal cancer (CRC) may constitute a novel antitumor approach (Cai et al.,2016). The another protein, which is encoded by the HCMV named UL138 found to have an important role in the establishment of the latency of the virus in some certain cells (Montag et al., 2011) .

In our study the UL138 gene was detected in the five patients with Non-Hodgkin's lymphoma, including two of 25 males and three of 25 females. Only one (4%) female patient with Non-Hodgkin's lymphoma was found to be

positive for IE1, while, in Hodgkin group only one (4%) male showed positive for UL 138 but no IE1 was detected.

The proteins pp65, IE1, US 28 are produced by HCMV and have been involved in numerous significant events in tumor progression (Ponticelli et al., 2011; Cai., 2016; Lucas., 2011). Pp65 is the most abundant virion protein and non-infectious viral particles that are assembled during active infection (Libard et al., 2014). Besides ,the detection of IE1 mRNA indicates the presence of active or reactivation of HCMV infection (Harwardt., 2016). High rate of 76% HCMV pp65 have been detected in biopsies of 29 patients with diffuse large B-cell lymphoma of the central nervous system (Libard., 2014) (CNS DLBCL). Subsequently high rate of 57%, 49% and 44% of HCMV pp65 Antigenemia have been detected in patients with Follicular and aggressive Non-Hodgkin lymphoma and with Hodgkin lymphoma respectively (Jacopo., 2014). While in our survey only one patient with subtype, diffuse large B-cell lymphoma was shown to be positive for IE1. It was exhibited the detection of HCMV UL138 gene is highly conserved in clinical strains and latency of HCMV infection (Grainger et al., 2010). Jinji et al., have detected UL138 in 11 of 58 (18.97%) of the patients with gastric cancer (Jinji et al., 2014). In our study, 5/25 (20%) of patients with Non-Hodgkin lymphoma showed positive for UL138, which is in accordance with Jinji et al finding (Jinji et al., 2014). In addition, the other latent human cytomegalovirus proteins, US28, UL133, UL135, UL136 have been investigated in various types of cancer tissues (Cai., 2016; Jinji et al., 2014).

The achievement of both UL138 and IE1 detection are cost effective and easily can be performed at

any common laboratory. Diagnosis of HCMV based on HCMV IgG does not clarify the exact status of HCMV infection. Although the presence of HCMV IgG seropositivity could be described as independent predictor of clinically relevant CMV infection (Drew et al., 2007). In our study the diagnosis of the HCMV activation replication was based on detection of IE1 mRNA by RT-PCR which is a qualitative test but to achieve highly sensitivity performance it requires quantification realtime – PCR assay. While the diagnosis of the latent HCMV was based on detection of UL138 mRNA by Nested-PCR which is also a qualitative test but to achieve highly sensitivity test it requires to fulfill quantification realtime – PCR assay. With the aforementioned data to improve treatment and management of HCMV infection, patients with Non-Hodgkin lymphoma should be screened for both UL138 mRNA and IE1 mRNA before and during chemotherapy.

The association of other viruses, including the Epstein Barr virus (EBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV) and hepatitis B (HBV) among the patients with non-Hodgkin's lymphoma have been reported (Economides et al., 2016; Hoffmann et al., 2016; Engels et al., 2010). The evaluation of the mentioned viruses have not been studied in the present study but it requires to be evaluated in the further studies.

Conclusion, UL138 mRNA as a predictor of HCMV latency and IE1mRNA as a predictor of activation or reactivation of HCMV have been detected in patients with Hodgkin and Non-Hodgkin lymphoma. Both detection of UL138 mRNA and IE1 mRNA were carried out by PCR which are qualitative tests but to achieve highly sensitivity performance it requires to fulfill quantification realtime – PCR assay. High rate of 20% expression of UL 138 mRNA have been detected in the patients with non-Hodgkin's lymphoma subtype T cell lymphoma, which indicates UL138 mRNA may play a possible role in development of Non-Hodgkin's lymphoma, however it requires further investigation.

However the evaluation of other CMV latent genes or proteins, including US28, UL133, UL135, UL136 should be investigated in cancerous patients. To improve treatment and to prevent consequences of HCMV infection, the screening of the both UL138 mRNA and IE1mRNA of HCMV should be implemented for patients with Hodgkin disease and Non-Hodgkin lymphoma before treatment and during chemotherapy.

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## Authors' Contributions

Study concept and design: Manoochehr Makvandi, Alireza Samarbafzadeh; acquisition of data: Hamide Mehravaran; analysis and interpretation of data: Manoochehr Makvandi and Hamide Mehravaran; drafting of the manuscript: Hamide Mehravaran, HadisKiani;

critical revision of the manuscript for portant intellectual content: Manoochehr Makvandi; statistical analysis: Manoochehr Makvandi and Hamide Mehravaran; Administrative, technical, and material support: Hamide Mehravaran, Niloofar Neisi, Seyedeh Zeinab Hoseini, Hashem Radmehr, Toran Shahani; Study supervision: Manoochehr Makvandi and Alireza Samarbafzadeh.

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## Financial Disclosures

The authors have no financial interest related to the material in the manuscript.

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