

## Association of HHV-6 with Hodgkin and non Hodgkin lymphoma

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### ABSTRACT

**Background and Objectives:** Human Herpes 6 virus (HHV-6) could remain latent and chronic in the host cells after primary infection. HHV-6 genome encodes certain transactivation proteins which may results in development of malignant lymphoma. The association of human herpes six virus (HHV-6) infection and Hodgkin and Non-Hodgkin lymphomas is strongly supported by epidemiological studies. The aim of this study was to determine the prevalence of HHV-6 among the patients with Hodgkin, Non- Hodgkin's lymphoma.

**Materials and Methods:** Overall 44 blocks of formalin-fixed, paraffin-embedded of the patients including 22(50%) Hodgkin and 22(50%) Non-Hodgkin Lymphoma were collected. Initially the section of 5µm-thickness were prepared from the formalin-fixed, paraffin-embedded tissue blocks. Then the deparaphinization was carried out for each sample. The DNA was extracted, followed by nested PCR for detection of HHV-6. Based on PCR product size and sequencing, the HHV-6 A or B subtypes were characterized.

**Results:** 12/22(54.54%) cases of Hodgkin and 8/22 (36.36%) Non-Hodgkin's lymphoma were shown as positive for HHV-6. Out of 12 positive HHV-6 in Hodgkin lymphoma, 10 patients (45.45%) belonged to variant A while 2 cases (9.09%) were found positive for both HHV-6A and HHV-6B. All the Non Hodgkin samples (n=8, 36.36%) showed positive for HHV-6 variant A.

**Conclusion:** High prevalence of HHV-6 was found among the patients with Hodgkin and Non-Hodgkin's lymphoma. Two patients with Hodgkin lymphoma had mixed HHV-6A and HHV-6B infections. It is recommended patients with Hodgkin and Non-Hodgkin should be screened for HHV-6 detection before chemotherapy.

**Keywords:** Nested PCR, Human herpes virus 6, Hodgkin lymphoma, Non-hodgkin lymphoma

### INTRODUCTION

Human herpes virus 6 (HHV6) was discovered in patients with lymph proliferative disorders in 1986 (1). HHV6 is categorized in β-subfamily of herpes (2). It is classified into two major subgroups HHV-6A

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and HHV-6B. Most of children are infected by one of two subgroup A or B before the age of two year. No clear disease have been defined for HHV-6A. HHV-6B is causative agent for exanthema subitum (roseola) meningitis, meningoencephalitis and hepatitis (3). HHV6 is a lymphotropic virus and replicates in CD4+T lymphocytes. Human Herpes 6 virus can establish latent and chronic infection after the primary infection. Both HHV-6A and HHV-6B have the ability to integrate their genome into the host chromosome. HHV-6 genes encode transactivation protein pDR7, which found to possess transforming capacity (4). The other protein pU94 was found to suppress Ras-induced transformation and might contribute to tumor genesis (5). HHV-6 is associated with some malignant disorders, including Hodgkin's disease, Non-Hodgkin's lymphoma, acute lymphoblastic leukemia and carcinoma (6). The Hodgkin's lymphoma disease (HD) is a common malignant neoplasm of germinal center B-cell origin worldwide and histopathologically characterized by the existence of very large Hodgkin Reed-Sternberg cell (HRS).

The annual incidence of HD is about 2-3/100000 people and accounts for about 1% of all cancers worldwide (7). The low prevalence of Hodgkin lymphoma have been reported in Iran (8). Although the etiology of Hodgkin lymphoma is unknown but the association of Epstein Barr (EBV) with Hodgkin lymphoma is well described (8, 9). Non-Hodgkin lymphoma or Non-Hodgkin disease are various group of blood cancers that comprise any kind of lymphoma except Hodgkin's lymphomas. Types of NHL diversify significantly in their severity, from slow growing to very aggressive types (10). The etiology of NHL is unknown, but predisposing factors for development of Non-Hodgkin lymphoma are attributed to age, sex, genetic and environmental factors (11). In the US, data from 2007-2011 has shown that there were about 19.7 cases of NHL and 6.3 deaths per 100,000 among adults every year. About 2.1 percent of men and women are diagnosed with NHL at some point during their lifetime, and there were around 530,919 people living with non-Hodgkin lymphoma (12). Globally, as of 2010, there were 210,000 deaths, up from 143,000 in 1990 (13).

The prevalence of Non- Hodgkin lymphoma is not precisely defined and very limited in Iran (14). Recent studies have revealed that HHV-6 contribute to the pathogenesis of a wide range of malignant lymphomas. A possible pathogenic role of HHV-6 in lymph

proliferative disorders has been emphasized by the ability of HHV-6 DNA to transform established NIH 3T3 cells, which in turn form rapidly growing and metastasizing tumors when injected into nude mice (15).

This study was conducted to determine the HHV-6A or B DNA detection from the biopsies of patients with Hodgkin and Non Hodgkin lymphoma in Ahwaz city, the capital of Khuzestan province, Iran.

## MATERIALS AND METHODS

A retrospective study was conducted on 44 blocks of formalin-fixed, paraffin embedded tissue samples including 22(50%) cases of Hodgkin and 22 (50%) cases of Non-Hodgkin's Lymphoma. The diagnosis of Hodgkin and Non- Hodgkin's lymphoma was approved by the pathologist. All block samples were collected from Imam Khomini and Shafa hospitals, Ahwaz City, Iran During 2001 to 2011.

**Deparaffinized tissue samples.** Initially the sections of 5µm thickness were prepared from the each block sample then the samples were deparaffinized by xylene and ethanol (Germany, Merk). To each microtube containing sample, xylene was added and kept at 45 °C for 15 min followed by centrifugation at 14000rpm. This stage was repeated. The supernatant was discarded and 1ml absolute ethanol was added to precipitate and stored at the room temperature for 10 min and then centrifuged at 14000 g for 1 minute. The supernatant was discarded. This process was repeated by adding 70% ethanol, followed the same condition. Finally supernatant was discarded and precipitate was heated at 65 °C for 5 min to vaporize the ethanol residue. The pellet was then used to extract DNA (16).

**DNA extraction.** High pure PCR template preparation kit (Roche, Germany) was used for the extraction of DNA. The extracted DNA was stored at -70 °C until used for PCR amplification.

**Nested-PCR amplification.** The following primers conserved for immediate-early gene for the both variant HHV-6A and HHV-6B were used in nested PCR assay (17). The first round of PCR was performed in 25µl PCR reaction mixture, containing

7µl of extracted DNA, 2.5µl PCR buffer 10X, 0.5 µl dNTP 10mM, 1U of Taq DNA Polymerase, 0.5 µM of each outer primers of forward (5'TTCTC-CAGATGTGCCAGGGAAATCC -3') and reverse (5'-CATCATTGTTATCGCTTTCCTCTC -3'). The PCR reaction mixture was subjected in thermocycler (Techne TC-5000, UK) and programmed with following condition one cycle: 94 °C for 5 min; and 35 cycles: 94 °C for 1 min; 57 °C for 1 min; 72 °C for 1min, final extension 72 °C for 10 min. The second round was carried out with 5µl of the first round product, under the same condition described previously with the set of inner primers Forward (5'AGTGACAGATCTGGGCGGGCCCTAATA-ACTT-3') and reverse (5'-AGGTGCTGAGTGAT-CAGTTTCATAACCAAA-3'). The expected sizes of the PCR products for the HHV-6A were 195bp and for HHV-6B was 423 bp.

**Gel electrophoresis.** The PCR products was observed on a 2% agarose gel and developed by Safe Stain under constant voltage at 100V. The result was observed under ultraviolet in transilluminator. The sizes of bands of product were compared with 100bp Ladder (Fermentas). To verify the results the PCR product of the each Positive samples was sequenced.

**Statistical analysis.** Data were statistically defined in terms of median and range or mean and standard deviation ( $\pm$  SD), frequencies. For comparing categorical data, Chi square (Fisher's exact) test was done. A *p* value < 0.05 was considered statistically significant. All statistical calculations were done using SPSS version 21 (SPSS Inc., Chicago, IL, USA)

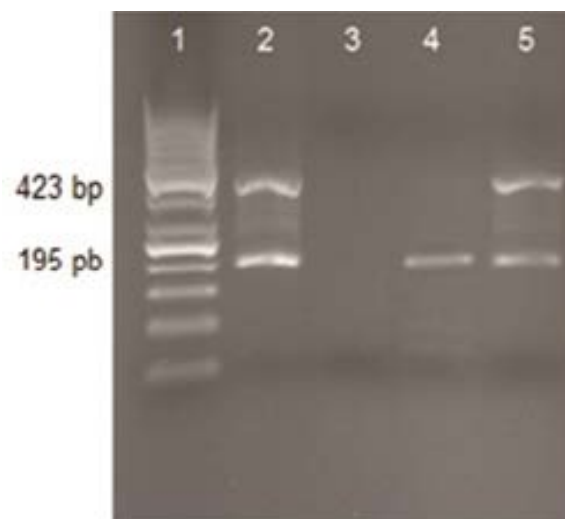
## RESULTS

22 Hodgkin lymphoma cases comprising 10/22 (45%) male and 12/ 22(54%) female with the mean age of 18.63 $\pm$ 13.36 years. Of those 22 Non-Hodgkin's lymphoma cases, 13 (59%) were male and 9 (40%) were female with the mean age of 37.68 $\pm$ 41.32 years.

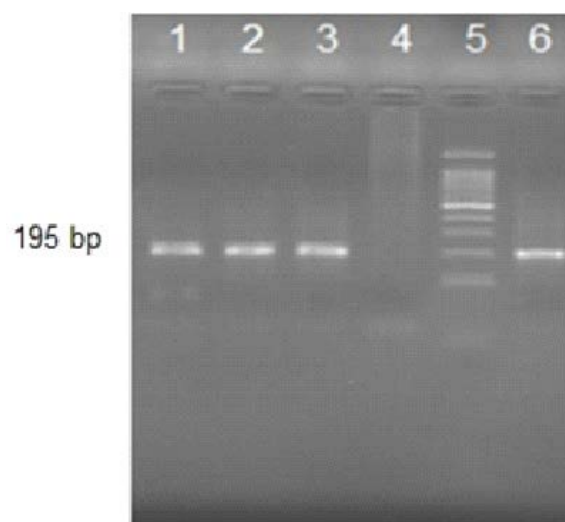
Of 22 of Hodgkin lymphomas 12 (54%) were positive for HHV-6 DNA (10 with HHV-6 A and 2 with both HHV-6A and HHV-6B)(Fig 1). Eight of 22 patients (36%) with Non-Hodgkin's Lymphoma were positive for HHV-6A only (Fig. 2). Overall high frequency of HHV-6 variant A (40.9%, 18 of 44 patients) were found among the both patients

with Hodgkin and Non-Hodgkin's Lymphoma.

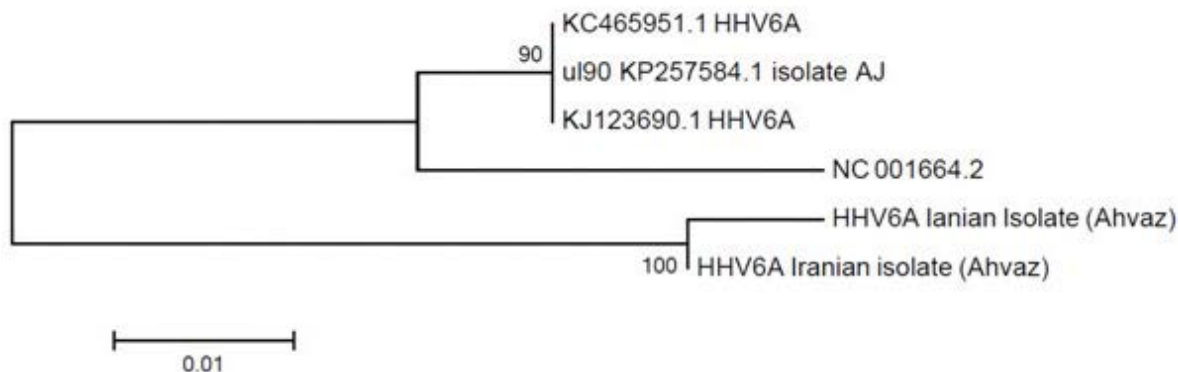
PCR products of 4 Hodgkin and 3 Non Hodgkin were sequenced and the resultants were aligned with standard references using Mega 6 then the Phylogenetic trees were drawn. The positive HHV-6 variant A and B were aligned and blasted separately using NCBI data base (Figs 3 and 4).



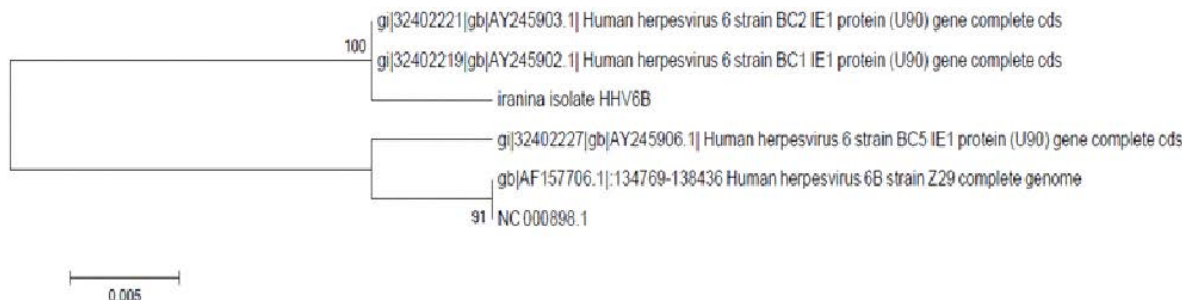
**Fig. 1.** Analysis of the mixed HHV-6 variant A and B Lane 1: 50-bp size marker Lane: 2 and 5 two control, Patients with Mixed HHV6 variant A and B



**Fig. 2.** Analysis of HHV6, variant A Lane 1-3 sample, Lane 4 Negative Lane 5: 100-bp size marker Lane 6 Positive control



**Fig. 3.** A phylogenetic tree constructed with Neighbor joining method and using the partial nucleotide sequences of UL 90 region HHV-6 A, Reference sequences were retrieved from GenBank with their accession numbers and origin indicated. 1000 replication bootstrap values.



**Fig 4.** A phylogenetic tree constructed with Neighbor joining method and using the partial nucleotide sequences of UL 90 region HHV-6 B, Reference sequences were retrieved from GenBank with their accession numbers and origin indicated. 1000 replication bootstrap values.

**DISCUSSION**

In our study 12 of 22 cases of Hodgkin lymphoma (54%) showed positive for HHV-6 DNA. Of these 12 cases, 10 were positive for HHV-6 A but 2 cases (9%) were positive for both HHV-6A and HHV-6B (Fig 1). On the other hand, 8 of 22 patients with Non-Hodgkin’s Lymphoma (36%) were positive for HHV-6A only (Fig 2). The rate of HHV-6 DNA detection by PCR in HD were reported controversial. Gledhii S et al. reported the absence of HHV-6 DNA in HD patients (18). Torelli BR et al. found low prevalence of HHV-6 DNA in HD cases (19). Valente G et al. described 73% of HD cases were associated with HHV-6 DNA (20). In study conducted by Schmidt CA et al. in Humborg, Germany, the frequency of HHV-6 DNA among 86 HD cases was 11/86 (13%), among

them 9.3% and 3.48% were HHV-6A and HHV-6B respectively (21). Our results is positioned between the results of high frequencies of 73% HHV-6 among the HD patients reported by Valente et al. (20), and the results of low frequency of 12% HHV-6 among the HD patients reported by Torelli et al (19).

The frequency of HHV-6 A or B in Non-Hodgkin’s lymphoma have been limited in Iran. Although the frequency of HHV-6 DNA in patients with neoplasm salivary gland was reported 18.9% in Esfahan city, Iran (22). Our results showed the frequency of HHV-6 virus in the patients with Non- Hodgkin lymphoma was 36.36% and all the detections were HHV-6A. The frequency of HHV-6A among the patients with Hodgkin lymphoma and Non-Hodgkin’s lymphoma was not significant ( $P = 0.351$ ).

The prevalence of HHV-6 DNA in patients with Non-Hodgkin's lymphoma is varied. Tailor et al. (2004) in Mumbai, had found high frequency of reactivated HHV-6 variant B (34%) among the patients with non-Hodgkin's lymphoma (23).

In our survey 2 (9.09%) patients with Hodgkin Lymphoma were co-infected with HHV-6 variants A and variant B. The distribution of HHV-6 among the Hodgkin lymphoma subtypes was 5 /9 nodular sclerosis (NS), 5/11 Mixed Cellularity (MC) and 2/2 lymphocytic predominance (LP). The distribution of HHV-6 among the subtype Non-Hodgkin lymphoma subtypes was 6/10 cases in Diffuse Large cell type and 2/7 cases in Burkitt lymphoma .

In conclusion, this study described high prevalence of HHV-6 A among the patients with Hodgkin and non-Hodgkin's lymphoma. HHV-6 is related to immunosuppressive therapy or transfusion of blood and blood products. The role of HHV-6 in the etiology of malignancy can not be clearly defined. This needs further assessment in large scale follow up studies. Thus, the initial screening of patients with hematological malignancies for HHV-6 might be considered mandatory.

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