Original Article

Relationship between Human Herpes Virus Type 6 and Childhood T-Cell Leukemia-Lymphoma

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Abstract

Background: Previous studies have pointed to the major role of viruses in the pathogenesis of cancers, especially lymphoproliferative cancers.

Objectives: The current study investigated the relationship between recent Human Herpesvirus Type 6 (HHV6) infection and childhood leukemia-lymphoma syndrome.

Methods: From January 2011 to December 2012, we entered every new case of acute lymphoblastic leukemia, non-Hodgkin lymphoma, acute myeloblastic leukemia, and Hodgkin lymphoma as the case group (n=48); moreover, 60 patients were randomly selected as controls from hospitalized children without infectious agents and myocarditis in the department of pediatric cardiology in Shiraz, Iran. Immunophenotyping of bone marrow or lymph node biopsy was performed for the case group. The DNA was extracted from all collected samples using a DNA extraction kit (Invitek Company, Germany), and a Real-time quantitative polymerase chain reaction (qPCR) for the U38 gene of HHV-6 was performed for the detection of the HHV-6 genome for both groups.

Results: In the case group, 48 patients with the age range of 0-18 years were assigned to four subgroups: 1- Acute Lymphoblastic Leukemia, 2- Acute Myeloblastic Leukemia, 3- Hodgkin Lymphoma, and 4- non-Hodgkin Lymphoma. In the case group, there were four positive HHV-6 PCR patients: One in Acute Lymphoblastic Leukemia, one in Non-Hodgkin Lymphoma, and two patients in the Hodgkin Lymphoma subgroup. None of the patients with T-cell leukemia-lymphoma had positive PCR. The frequency of HHV6 PCR positive was not significantly different between the case (8.3%) and control (1.6%) groups (P=0.169). No HHV-6 PCR positive was detected in T-cell leukemia/lymphoma patients.

Conclusion: As evidenced by the results of the present study, HHV-6 infection has no significant difference in children with T-Cell leukemia-lymphoma and healthy people.

Keywords: Human Herpesvirus type-6, T-cell leukemia-lymphoma

1. Background

Hematologic malignancies are among common childhood cancers; for instance, leukemia and lymphoma comprise more than 40% of the cancers in children below 15-year age. Acute Lymphoblastic Leukemia (ALL) is the most prevalent cancer in this age group (25.4%) (1). The HHV-6 is a human herpesvirus that tends to invade CD4+ lymphocytes. After infection, this virus remains latent in T-cells but can reactivate under certain conditions (2). In the acute phase after the infection, the virus can be isolated from the peripheral blood and saliva lymphocytes (3). In addition, a specific antiviral antibody that is transferred vertically at birth leads to immune protection for several months after birth. Nonetheless, when the antibody titer decreases during the first year after infection, the incidence of the infection increases with its highest rate at the ages of 6-24 months (4).

All children have positive serology before the age of four; nonetheless, this virus can merge with the patients' chromosomes and result in the hereditary transmission of DNA. Moreover, HHV-6 viral DNA can remain in the blood and lymphocytes for a long time (4). The HHV-6 can be transmitted through saliva, primarily infecting the oropharynx and regional lymph, finally invading mononuclear cells (5). Previous studies have demonstrated that viruses have a major role to play in the pathogenesis of cancers, especially lymphoproliferative cancers. The prevalence of ALL in lower ages is related to the time of development of the immune system when it has the highest vulnerability to the oncogenic effects of specific viruses (6).

Furthermore, some studies have pointed to an increased risk of ALL in children whose mothers were infected with influenza, varicella, and other viruses. Nevertheless, no significant relationship has been demonstrated between the risk of ALL and viral exposure in delivery (7). The seasonal prevalence of ALL can also be associated with higher viral exposure; however, no relationship has been observed yet (6). Overall, the relationship of HHV-6 with T-cell ALL and T-cell lymphoma is still controversial.

2. Objectives

In light of the aforementioned issues, the present

study aimed to investigate the relationship between recent Human Herpesvirus Type 6 (HHV6) infection and childhood leukemia-lymphoma syndrome.

3. Methods

3.1. Study design and participants

The present study was conducted on patients under the age of 18 years with the primary diagnosis of ALL, non-Hodgkin lymphoma (NHL), acute myeloblastic leukemia (AML), and Hodgkin's lymphoma hospitalized in Amir Oncology Hospital, affiliated with Shiraz University of Medical Sciences from April 2011 to March 2012. In all, 48 patients and 60 controls subject were recruited for the study. In addition to obtaining bone marrow samples for cytology, flow cytometry, and cytogenetic for the diagnosis of the type of cancer, a 2ml blood sample was drawn, added to a test tube containing oxalate as an anticoagulant for performing HHV-6 PCR, and sent to the Microbiology Research Center in 2 hours. The patients who were newly diagnosed with NHL and Hodgkin's lymphoma and were referred for the investigation of bone marrow involvement and chemotherapy were also enrolled in the study after bone marrow aspiration. Bone marrow aspiration was performed in the operating room or hospital emergency department under completely sterile conditions. The inclusion criteria were as follows: age range of under 18 years, the definite pathological diagnosis of NHL, AML, ALL, and Hodgkin's lymphoma, and transfer of the bone marrow sample before the beginning of chemotherapy. On the other hand, the exclusion criteria were having normal bone marrow samples and pathological diagnosis of cancers other than those mentioned above. The control group was randomly selected from the patients hospitalized in the department of pediatric cardiology due to cyanotic heart disease or the performance of angiography. The inclusion criterion of the control group was the absence of infectious agents and myocarditis. After obtaining written informed consent from the parents, a 2cc peripheral blood sample was obtained from the control group participants, added to a test tube containing oxalate as an anticoagulant, and sent to a microbiology laboratory for HHV-6 PCR. From April 2011 to March 2012, 74 patients underwent bone marrow aspiration. Among these patients, 26 cases were excluded from the study due to the diagnosis of other hematologic neoplasms, such as aplastic anemia, idiopathic thrombocytopenic purpura(ITP), and normal bone marrow aspiration.

3.2. Outcome measurement and HHV-6 Detection
3.2.1. DNA extraction

The DNA was extracted from all collected samples using a DNA extraction kit (Invitek Company, Germany) according to the manufacturer's instructions.

3.2.2. Real-time quantitative polymerase chain reaction (qPCR) for HHV-6

Real-time qPCR for the U38 gene of HHV-6 was performed as described elsewhere ([8]), with one modification. The forward primer used for these experiments was 5′-TGCTTCTGTAACGTGTCTTGGA-3′ (sense) and contained one less adenine molecule on the 3′ ends than the published primer. In brief, the samples positive for HHV-6 DNA by qualitative PCR were purified using the Wizard SV Genomic DNA purification system (Promega) and were concentrated ~10-fold, and the DNA concentration was measured spectrophotometrically. Each sample was run in triplicate. The detection limit of this assay is 10 copies. Mean values were used if results were available from two or more wells, and the results were reported as copies of HHV-6 per microgram of DNA. If two or more wells did not have measurable copy numbers, the result was set at 5 copies/µL since the qPCR was performed only on samples that had a positive reaction in the qualitative PCR, which was able to detect <10 copies

3.3. Statistical analysis

Data were analyzed in SPSS software. Descriptive data were presented as frequency and percentages. The Chi-square test and Fisher's exact test were employed for the comparison of the qualitative data between the two groups or among different groups.

4. Results

In the present study, the patients were assigned to four groups of ALL (B-cell Precursor, T-cell), AML, NHL (T-cell, B-cell), and Hodgkin's lymphoma, as well as three age groups: below one year, 1-10 years, and 10-18 years. Gender distribution was also determined in all the study groups (Tables 1 and 2).

According to the study results, out of the 48 patients, 31 (64.6%) cases had ALL, among whom 25 patients had B-cell Precursor ALL, and 6 cases had T-cell ALL. Moreover, 5 (10.4%) patients had AML. In addition, 8 (16.7%) cases had NHL, among whom seven patients had B-cell NHL, and one subject had T-cell NHL. Finally, 4 (8.3%) patients had Hodgkin's lymphoma. The highest frequency of the patients was related to the 1-10-year age group (n=35, 72.9%), while the lowest frequency pertained to the below-1-year age group (n=1). Considering gender distribution, 30 (62.5%) patients were male, with a male-to-female ratio of 1/7.

Among the patients, only four cases showed positive PCR (one with B-cell Precursor, one with B-cell NHL, and two with Hodgkin's lymphoma). However, none of the patients with T-cell ALL and T-cell NHL demonstrated positive PCR (Table 3). In two cases with Hodgkin's lymphoma, an abnormally high level of HHV-6 DNA was detected (approximately 100,000 NVCs; median of 130,150 NVCs), while in the

Table 1. Comparison of gender distribution in the patient and control group

Variable		Gender		Total
		Male	Female	
Group	Patient	30 (62.5%)	18 (37.5%)	48 (100.0%)
	Control	31 (51.7%)	29 (48.3%)	60 (100.0%)
Total		61 (56.5%)	47 (43.5%)	108 (100.0%)

Table 2. Comparison of age distribution in patient and control group

Variable		Age (years)			Total
		<1	1-10	10-18	
Group	Patient	1 (2.1%)	35 (72.9%)	12 (25.0%)	48 (100.0%)
	Control	4 (6.7%)	35 (58.3%)	21 (35.0%)	60 (100.0%)
Total		5 (4.6%)	70 (64.8%)	33 (30.6%)	108 (100.0%)

rest of the positive patients, the median HHV-6 DNA load was only 1.81 NVCs.

The positive cases in this study were:

1. A 14-month-old boy with Pre-B ALL and 1000 copies in Real-Time PCR in both peripheral blood and bone marrow samples,

2. A 6-year-old girl with B-cell NHL and lower than 300 copies in the peripheral blood sample,

3. A 3-year-old girl with Hodgkin's lymphoma and 1350 copies in the bone marrow sample and 500 copies in the peripheral blood sample,

4. A 15-year-old girl with Hodgkin's lymphoma and lower than 500 copies in the peripheral blood sample. In the control group, only a 14-month-old girl who had been hospitalized for angiography had positive PCR with 3492 copies.

Table 3. Comparison of polymerase chain reaction results of the patient and control group

Variable		Acute Lymphoblastic Leukemia		Acute myeloblastic leukemia n=5	Non-Hodgkin lymphoma		Hodgkin lymphoma n=4	control n=60
		B cell Precursor n=25	T cell Precursor n=66		B cell Precursor n=7	T cell Precursor n=1		
Polymerase chain reaction	**Positive**	1	0	0	1	0	2	1
	Negative	24	6	5	6	1	2	59

5. Discussion

As evidenced by the results of this study, none of the patients with T-cell ALL and T-cell NHL showed positive PCR. In addition, the rate of PCR-positive cases was 8.3% in the patient group and 1.6% in the control group, and the difference was not statistically significant. In a study in 2002, Salonen et al. investigated 40 patients with ALL and AML regarding the amount of HHV-6 IgG and IgM. The study results illustrated that 97.5% of the patients had IgG and 40% IgM against HHV-6, while these percentages were 92.3% and 7.7% in the control group.

The high rate of IgM in the patient group indicates recurrent infection or response to the primary infection. Virus-specific IgM appears in the second week of HHV-6 infection and remains for 2-3 months, while IgG remains for a longer period. The findings of the study by Salonen proposed HHV-6 as the etiology of leukemia. Nonetheless, since IgG was high in both study groups, high IgM might have resulted from virus reactivation. Yet, it cannot be determined whether virus reactivation led to leukemia or vice versa (9).

In another study, Shiraizu examined 47 patients with Hodgkin's lymphoma through the assessment of PCR-HHV-6 in the pathological sample. The results of the mentioned study indicated that none of the patients had positive PCR (10). However, the latent cases with a lower number of copies were expected to be observed. The obtained results can be ascribed to the low sensitivity of the PCR kit utilized in the refereed study (a copy of 2000 cells). Furthermore, in addition to the tissue sample, they should have simultaneously used the peripheral blood sample for PCR evaluation so that the latent cases, particularly in mononuclear cells, would be identified. Nevertheless, the identification of latent cases could not prove an association between HHV-6 and Hodgkin's lymphoma.

In another study by Loutfy et al., 46% of the patients with HL and NHL showed positive PCR for cytomegalovirus (CMV) or HHV-6, which was significantly higher compared to the control group. In addition, HHV-6 DNA was positive in 32% of the patients. Since this investigation was performed on lymphoid tissue, its positivity indicated that the virus

had been activated in this tissue (11). In general, the study findings suggested that HHV-6 was responsible for the cellular immune deficiency factor leading to CMV reactivation or reinfection, and both viruses caused acute lymphoma in the lymphoid tissue. Consequently, HHV-6 showed to have synergistic effects on CMV infection.

In the same context, the study by Seror et al. pointed out that 14% of the ALL patients had positive PCR at diagnosis, while 34% showed positive PCR at remission. In addition, the viral load was higher at remission in comparison to the time of diagnosis. That study was the first research performed on the viral load in ALL patients at both diagnosis and remission times (12). The high number of positive cases and high viral load at remission might be due to virus reactivation following chemotherapy and the reduction of the patient's immunity level.

The findings of the present study revealed that HHV-6 was able to invade bone marrow progenitors but could not infect the leukemic cells. The HHV-6 involves differentiated cells (lymphocytes) and immature cells (early bone marrow progenitors CD34+) but cannot involve averagely differentiated cells (lymphocytes). This theory was proposed by Andre-Garnier in 2004. Nonetheless, since the early stages of leukemogenesis might last for several months before the diagnosis and begin with a chromosome translocation, the role of this virus in hematologic neoplasms cannot be rejected. Hobacek et al. assessed the probability of chromosomal integration of this virus in children suffering from ALL and AML. According to their reported results, out of 399 patients, 5 (1.5%) cases had chromosomal integrity of this virus. Therefore, no significant difference was observed between the children with leukemia and the healthy population regarding the prevalence of HHV-6 chromosomal integrity (13).

The study by Arzanian and Shahriari pointed out that T-cell ALL comprised 31.9% of the total of ALL cases in Iran (14), which is twice as much as the global average. In the present study, 19.3% of all the cases with ALL were T-cell originated, which is closer to the global average. Therefore, by evaluating bone marrow samples at the acute stage of the disease before chemotherapy and employing the accurate Real-Time PCR method, we expected to have more positive cases in this study.

5.1. Study Limitations

In the present study, the number of positive cases in the patients and the control group was too low to confirm or reject the role of this virus in the pathogenesis of hematologic neoplasms. Moreover, the higher number of positive cases with Hodgkin's lymphoma cannot be taken into account due to the small number of patients and the fact that the patient's bone marrow involvement was negative. Therefore, it was better to perform PCR on the tumor pathological samples in the cases suffering from Hodgkin's lymphoma and NHL, and the positivity of the tissue samples would reveal a more logical relationship with the pathogenesis of the virus. However, since the patients with lymphoma were referred after pathological diagnosis, the evaluation of the initial sample or performance of another biopsy was not possible.

6. Conclusion

The results of the present study pointed out that HHV-6 infection has no significant difference in children with T-Cell leukemia-lymphoma and healthy people. For more accurate conclusions, similar studies with a larger sample size are recommended to be conducted.

Acknowledgments

Footnotes

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