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Prevalence of Human Herpesvirus 8 DNA in Peripheral Blood Mononuclear Cells of Acute and Chronic Leukemia Patients in Taiwan

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December 07, 2010

Dear Dr. Alfredo Garzino-Demo,

Thank you very much for giving the minor revision decision to our manuscript entitled "**Prevalence of Human Herpesvirus 8 DNA in Peripheral Blood Mononuclear Cells of Acute and Chronic Leukemia Patients in Taiwan** ", which has been previously assigned as Manuscript id: FEMSIM-10-08-0184.R1. Our manuscript has been revised according to reviewer's comments.

Thanks very much again!

Sincerely,

Cheng-Wen Lin, PhD
Professor
Department of Medical Laboratory Science and Biotechnology
China Medical University

The responses to the comments of Reviewer #1 are listed in the following:

Q1. There are no comments

Ans 1.: Thanks.

The responses to the comments of Reviewer #2 are listed in the following:

Q1. What the reviewer has asked is the lowest copy number that can be detected by the assay.

Ans 1.:

We have cited the reference (Tisdale et al., 1998) in the Page 4 in the revised manuscript: e.g., HHV-8 DNA was detected based on a 233- and a 160-bp fragment of ORF-26 using the nested PCR reactions that could detect less than 3 copies of HHV-8 (Tisdale et al., 1998).

The responses to the comments of Reviewer #3 are listed in the following:

Q1. The abstract says that "the prevalence of HHV-8 DNA in peripheral blood mononuclear cells (PBMCs) in Taiwanese leukemia populations has not been investigated", yet in the main body, it says the incidence was similar to the percentage of HHV-8 DNA in PBMCs among the general population in Taiwan and quotes Lin et al., 2008. The manuscript by Lin et al, however, only measures DNA in HIV infected patients. It is thus difficult to draw any useful conclusions from this study.

Ans 1.:

We mentioned that "In this study, the percentage of HHV-8 DNA in PBMCs of the relatives' cases (8.94%, 11/123) and leukemia patients (10.29%, 14/136) was similar to our previous research in that 8.9% of HIV-infected Taiwanese patients had the seropositivity of HHV-8 DNA detected in plasma (Lin et al., 2008)." in the Page 6 in the revised manuscript. In addition, we concluded that "In conclusion, we have demonstrated the prevalence of HHV-8 DNA in PBMCs in Taiwanese leukemia patients (10.29%) and their relatives (8.94%).

But, there is no significant difference between leukemia patients and their relatives, which is probably associated with environments. In Taiwan, the prevalence of HHV-8 DNA in PMBCs of leukemia patients does not correlate with any type of leukemia like ALL, AML, CML and MM. This result can be applied in further epidemiological studies." in the Page 7 in the revised manuscript.

Prevalence of Human Herpesvirus 8 DNA in Peripheral Blood Mononuclear Cells of Acute and Chronic Leukemia Patients in Taiwan

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Abstract

Human herpesvirus 8 (HHV-8) is associated with the development of Kaposi's sarcoma and several other human malignancies. The prevalence of HHV-8 DNA in peripheral blood mononuclear cells (PBMCs) in Taiwanese leukemia populations has not been investigated. In this study, HHV-8 DNA was extracted from peripheral blood PBMCs, being detected in 10.29% of the leukemia cases and 8.94% of the relatives' cases. In addition, the prevalence of HHV-8 DNA in PBMCs was nonsignificantly associated with gender, age and leukemia subtypes. The study examines the prevalence of HHV-8 DNA in PBMCs in Taiwanese leukemia and can be applied in further epidemiological studies.

Keywords

Human herpesvirus 8; leukemia, peripheral blood mononuclear cells

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), belongs to the gammaherpesvirus (Lin et al., 2008; Ablashi et al.,2002). HHV-8 causes KS, multicentric Castleman disease and primary effusion lymphoma (Ablashi et al., 2002; Haddad et al., 2008). Epidemiological findings suggest that the prevalence of HHV-8 infection is higher in immunodeficient individuals and homosexual men (Blackbourn et al., 1999). Compelling evidence has suggested that the virus might be associated with two other B-cell lymphomas, primary effusion lymphoma and multicentric Castleman'disease (Duprez et al., 2004). The HHV-8 genome contains several genes capable of causing oncogenic transformation and immunological disturbances (Thirunarayanan et al., 2007). Viral oncogenesis seems to depend on other cofactors such as cytokine dysregulation and the functional state of the immune system (Samaniego et al., 2002). The prognosis for KSHV/HHV8-associated lymphomas is poor (Carbone et al., 2008). The exact oncogenic mechanisms of KSHV/HHV8 are not clearly defined. Patients with lymphoma, leukemia, autoimmune cytopenias and myeloproliferative disorders had a higher seropositive for HHV8 IgG antibody than in general population in Taiwan (Tsai et al., 2005), but the prevalence of HHV-8 DNA in peripheral blood mononuclear cells (PBMCs) in Taiwanese leukemia populations has not been

investigated.

This study was based on leukemia patients and their relatives as control. The subjects examined were 259 people (136 patients and 123 relatives) recruited from hospitals of China Medical University. Diagnosis was made based on standard morphologic examination and cytochemical analysis of the WHO classifications (Harris et al., 2000). HHV-8 DNA in PBMCs was extracted using Blood Genomic DNA Extraction Kit and detected by nested PCR protocols that amplified the KS330 region of ORF-26 using the set of primers used in the first identification of herpesvirus-like sequences in KS (Chang et al., 1994). The presence of KSHV/HHV8 evaluated by DNA analysis has been demonstrated in KS lesions from all risk groups worldwide and is essential for the development of KS (Carbone et al., 2008). The sensitivity of this primer pair was also evaluated and determined by the recombinant protein-based Western blot strip assay in our laboratory, showed higher than 95% (Yang et al., 2009). HHV-8 DNA was detected based on a 233- and a 160-bp fragment of ORF-26 using the nested PCR reactions that could detect less than 3 copies of HHV-8 (Tisdale et al., 1998). PCR products were detected by electrophoresis with ethidium-bromide staining. Result variables were explored for their association with HHV-8 status at p <0.10 and considered for inclusion in the regression model. The contribution to the models by other potential confounding

variables was tested by means of the likelihood ratio test. Unconditional logistic regression was used to estimate the odds ratios (OR) and 95% confidence interval (95% CI) in order to measure the association between specific variables and the risk of leukemia.

No differences in positive rates of HHV-8 DNA in PBMCs were observed in the distribution of cases in relation to age and sex (Table 1). Males and females presented similar seropositivity rates (9.02% and 10.32% respectively) (OR= 0.86, 95% CI= 0.38-1.97). Moreover, the subjects were placed into four main age groups. Prevalence of HHV-8 DNA in PBMCs was similar at all ages, but the group between the ages of 16–30 years was associated with a non-significantly increased risk (OR=2.69, 95% CI= 0.76-9.56). Our finding correlated with the previous report in that no difference in the seropositive rate is associated with gender or age in patients with lymphoma, leukemia, autoimmune cytopenias and myeloproliferative disorders (Tsai et al., 2005). However, our finding was not in agreement with the association of male gender with prevalence of plasma HHV-8 DNA in HIV-infected patient (Lin et al. 2008). Moreover, higher prevalence of plasma HHV-8 DNA in HIV-infected patients was in younger than 40 years old (Lin et al., 2008). The difference could be due to the risk of HHV-8 transmission by the sexual contact and intravenous injection routes in HIV patients.

HHV-8 DNA in PBMCs was detected in 10.29% (14/136) of the leukemia cases and 8.94% (11/123) of the relatives' cases. The prevalence of HHV-8 DNA in PBMCs was found to be not significantly different between these two groups (p = 7.31). Overall, 14 (10.29%) of the Taiwanese leukemia patients had HHV-8 DNA detected in PBMCs, higher than the percentage (5.8%, 29/501) of plasma PCR-positive HHV-8 cases among malignant lymphoma patients in Spain (de Sanjosé et al., 2004). In this study, the percentage of HHV-8 DNA in PBMCs of the relatives' cases (8.94%, 11/123) and leukemia patients (10.29%, 14/136) was similar to our previous research in that 8.9% of HIV-infected Taiwanese patients had the seropositivity of HHV-8 DNA detected in plasma (Lin et al., 2008). Among the major leukemia subtypes, prevalence of HHV-8 DNA in PBMCs was non-significantly associated with acute lymphoid leukemia (OR=0.84, 95% CI= 0.32-2.18), acute myeloid leukemia (OR=1.05, 95% CI= 0.28-3.98), chronic myeloid leukemia (OR=0.59, 95% CI= 0.07-5.35), aplastic anemia (OR=0.88, 95% CI= 0.10-7.46) and solid tumors (OR=0.39, 95% CI= 0.04-3.83). HHV-8 infection was not found in patients with multiple myeloma. The detection of HHV-8 DNA in malignant lymphoma showed that no overall differences in the HHV-8 prevalence could be found between cases and controls. The results did not correlate with the high frequency of HHV-8 positivity in patients with malignant B cells like chronic

lymphocytic leukemia (28.6%) and acute lymphoblastic leukemia (18.8%) (Hermouet et al., 2003). In addition, HHV-8 was associated with two subgroups: lymphoplasmacytic lymphoma and B-cell lymphoma (de Sanjosé et al., 2004). In this connection, the detection in peripheral-blood mononuclear cells showed that the copy number was not distinguished between patients with AIDS-associated Kaposi's sarcoma and AIDS-associated non-Hodgkin's lymphoma using real time PCR (Lin et al., 2009). In addition, variable HHV-8 seroprevalence rates among HIV-infected patients have been reported, for example, 55% in Taiwan (Yang et al., 2009), 63% in Uganda (Hladik et al., 2003), 48% in Greece (Panayiotakopoulos et al., 2005), and 86.7% in Spain (Gambus et al., 2001). Therefore, the HHV-8 prevalence in leukemia patients could be responsible for different worldwide populations.

In conclusion, we have demonstrated the prevalence of HHV-8 DNA in PBMCs in Taiwanese leukemia patients (10.29%) and their relatives (8.94%). But, there is no significant difference between leukemia patients and their relatives, which is probably associated with environments. In Taiwan, the prevalence of HHV-8 DNA in PMBCs of leukemia patients does not correlate with any type of leukemia like ALL, AML, CML and MM. This result can be applied in further epidemiological studies.

Acknowledgements

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Table 1.

Positivity for HHV-8 DNA in PBMCs according to gender, age and leukemia subtypes

	Total, N	N^+(%)	OR* (95% CI*)	p-value
Gender				0.724
Females	133	12(9.02)	reference	
Males	126	13(10.32)	0.86(0.38,1.97)	
Age (years)				0.359
<16	121	15(12.40)	reference	
16~30	59	3(5.08)	2.69(0.76,9.56)	
31~45	40	3(7.50)	1.78(0.49,6.40)	
>45	25	2(8.00)	1.66(0.36,7.67)	
Disease status				0.964
Controls	123	11(8.94)	reference	
ALL^+	76	8(10.53)	0.84(0.32,2.18)	
AML^+	35	3(8.57)	1.05(0.28,3.98)	
CML^+	7	1(14.29)	0.59(0.07,5.35)	
MM^+	3	0(0)	-	
AA^+	10	1(10.00)	0.88(0.10,7.64)	
Solid tumors^{++}	5	1(20.00)	0.39(0.04,3.83)	

* N, number; N^+, number of HHV-8 DNA positive; OR, odds ratio; CI, confidence interval;

$^+$ ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; AA, aplastic anemia

$^{++}$ Solid tumors including colon, lung, and nasopharyngeal tumor