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DETECTION OF HUMAN HERPESVIRUS 6 IN PATIENT WITH ACUTE MYELOID LEUKEMIA IN BAGHDAD-IRAQ

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ABSTRACT
Human herpesvirus 6 (HHV-6) is the causative agent of roseola infantum, as other herpesviruses; it can produce latent infection and also can integrate into the host DNA. Some research suggested that HHV-6 may have a role in the presence of some malignancies such as acute myeloid leukemia (AML). This cross-sectional case control study was conducted in National Center for Hematological Diseases (NCHD) at Al-Mustansyria University and Baghdad Teaching Hospital (BTH) in Baghdad-Iraq from September 2013 till November 2014. Thirty patients with AML from those attending NCHD and BTH were enrolled before starting anti-cancer therapy. They were 10 (33.3%) male and 20 (66.7%) female. The age range was 15-80 years. The diagnosis of AML was based on hematological and histopathological criteria. 59 healthy individuals were included as control groups. They were randomly selected from those unpaid blood donors attending the National Blood Bank-Baghdad. 30 (65%) were males and 29 (35%) were females. The age range was 18-59 years. The seropositivity rate of HHV-6 IgG and IgM (Abnova, Taiwan) antibodies were detected using enzyme linked immunosorbent assay (ELISA), and the indirect immunoflourescent assay (IFAT) (VIDIA, Czech Republic) was used for detection of anti-HHV-6 IgG, beside that, molecular detection and determination of plasma viral DNA load was achieved by quantitative polymerase chain reaction (qPCR) (DIA. PRO Diagnostic Bioprobes, Italy). All data were statistically analyzed, and p values < 0.05 were considered significant. The results showed a significant increase of anti-HHV-6 IgG seropositivity rate by IFAT in patients versus controls (83.3% *vs* 61.0%, p=0.032). Using the ELISA technique, the anti-HHV-6 IgG seropositivity rate was significantly higher among patients compared to controls (96.7% *vs* 72.9%, p= 0.007). Additionally, the anti-HHV-6 IgM seropositivity rate among patients was significantly higher compared to controls (36.7% *vs* 6.8%, p= 0.0004). HHV-6 DNA was detected in one patient with AML (3.3%), but not detected in any of the controls (0%). The Plasma HHV-6 viral load for the positive patient was $1.8x10^5$ particles/ milliliter. A significant association of anti HHV-6 antibodies among patients with acute myeloid leukemia was found suggesting that the virus may have a role in the development of the disease.

KEYWORDS: Human herpesvirus -6, acute myeloid leukemia, Roseola infantum.

INTRODUCTION

Human herpesvirus-6 is the smallest of herpesviruses about (170 kb) belong to beta-herpesvirus, and is one of the most widespread herpes viruses. HHV-6 primary infection generally occurs during the first 2 years of life, develops into roseola infantum (Yamanishi *et al*., 1988). HHV-6 has two very similar variants HHV-6A and HHV-6B that finally divided into 2 subtypes (Maeki and Mori, 2012). Transmission is most frequently through the shedding of viral particles into saliva and less frequently through by genital secretions. Several findings suggest that salivary gland tissues are a site of replication and a potential site for HHV-6 persistence (Fox *et al*., 1990; Arbuckle *et al*. 2011). However, HHV-6 can present in latent state at different anatomic sites (Dzieciątkowski *et al*., 2008). During early to mid-1990s, the first report of the presence of a partial or full-length integrated genome of HHV-6 in the DNA of peripheral blood mononuclear cells (PBMCs) was documented (Luppi *et al*., 1998). The Integrated HHV-6 that can be inherited from either parent has been recently confirmed to be activated in immunocompromised patients with pathogenic effects on the human body (Endo *et al*., 2014). HHV-6 induces immune dysregulation puts patients with chronic active infections at risk for autoimmune disease and certain lymphoproliferative disorders. Several investigators have suggested that HHV-6 also may be an oncogenic virus as cells transfected with HHV-6 have been shown to cause tumors in nude mice (Puri *et al.*, 1991). Acute myeloid leukemia makes large numbers of abnormal immature white blood cells which are derived from a myeloid stem cell in the bone marrow. A higher titers of HHV-6 antibodies were found in patients with AML, but not with acute lymphoblastic leukemia (ALL), suggesting an association between HHV-6 and AML (Gentile *et al* 1999). These findings actually confirmed previous study documented a higher HHV-6 seroprevalence and antibody titers in patients with acute myeloid leukaemia (Clark *et al* 1990).

PATIENTS & METHODS

This prospective cross-sectional case control study was carried out at the National Center of Hematological Diseases at Al-Mustansyria University and Baghdad

Teaching Hospital in Baghdad Iraq from September 2013 till November 2014. The study involves 30 patients, 10 (33.3%) male and 20 (66.7%) female before they started anti-cancer therapy. The AML was diagnosed according to hematological and histopathological criteria. Additionally, 59 apparently healthy individuals were included as a control group. The age and gender ratio were similar in the two study groups: the age range of patients was 15-80 years and for the controls was 18-59 years. The controls had no history of chronic illness and/or acute infection or under medication at the time of enrollment. All patients underwent blood tests and lymph node and bone marrow biopsy in order to diagnose and characterize the hematologic malignancy. The AML was diagnosis was based on standard hematological and histopathological criteria. Ten milliliters of venous blood were aspirated aseptically from patients and controls. Each blood sample was divided in two parts; first with EDTA anticoagulant tube, and the second in plane tube for separation of plasma and serum respectively. Both serum and plasma sample were stored in aliquots at -80°C till use. All the biochemical parameter was done by routine laboratory methods unless otherwise stated. The sera of all patients and control were tested for anti-HHV-6 IgG and IgM antibodies using ELISA kits (Abnova Company - Taiwan) and by Indirect Immunofluorescent (kit VIDIA Company from Czech Republic) for detection of anti-HHV-6 IgG antibody. The real -time PCR (Genetic PCR Solutions TM from Spain) was used for the detection of HHV-6 A-B genome. Another qPCR kit was used for quantification of HHV-6 plasma DNA viral load (DIA.PRO Diagnostic Bioprobes, Italy). All data were statistically analyzed using SSPS version 22, and P values less than 0.05 were considered significant.

RESULTS

Results in table (1) showed that the anti-HHV-6 IgG positivity rate among patients with AML and controls using the IFAT. 25 (83.3%) patients and 36 (61.0%) controls were positive. The difference between the two groups was statistically significant (p=0.032).

TABLE 1: Number and percentage of anti-human herpesvirus-6 IgG in acute myeloid leukemia group compared to control group by IFAT test

IFAT	AML		Control	
	No.	Percent %	No.	Percent %
Positive	25	83.3	36	61.0
Negative	5	16.7	23	39.0
Total	30	100	59	100
P=0.032 significant difference between proportions using Pearson Chi-square test at 0.05 level.				

The anti-HHV-6 IgG among patients with AML and control using ELISA was shown in table (2). 29 (96.7%) patients and 43 (72.9%) controls were positive. The difference between the two groups was statistically significant (p= 0.007).

TABLE 2: Number and percentage of anti-HHV6 IgG in acute myeloid leukemia group compared to control group by ELISA

ELISA	AML		Control	
	No.	Percent %	No.	Percent %
Positive	29	96.7	43	72.9
Negative	1	3.3	16	27.1
Total	30	100	59	100
P=0.007 significant difference between proportions using Pearson Chi-square test at 0.05 level.				

Table (3) revealed that the ELISA results of anti-HHV6 IgM positivity rate among patients with AML (36.7%) was significantly higher compared to controls (6.8%), (p= 0.0004).

TABLE 3: Number and percentage of anti-HHV6 IgM in acute myeloid leukemia group in compared to control group by ELISA

ELISA-IgM	AML		Control	
	No.	%	No.	%
Positive	11	36.7	4	6.8
Negative	19	63.3	55	93.2
Total	30	100	59	100
P=0.0004 (Significant difference between proportions using Pearson Chi-square at 0.05 levels.				

Table (4) revealed that the HHV-6 was detected in only one patient with AML (3.3%), while the virus was not detected in any of the controls (0%). The Plasma HHV-6 viral load for the positive sample was $1.8x10^5$ particles per milliliters as calculated by q PCR.

TABLE 4: Number and percentage of HHV-6 in acute myeloid leukemia group compared to control group by PCR

PCR	AML		Control	
	No.	%	No.	%
Positive	1	3.3	0	-
Negative	29	96.7	59	100
Total	30	100	59	100

DISCUSSION

Owing to absence of previous studies on the HHV-6 infection rate among Iraqi patients with hematological malignancies, actually this is a part of large comprehensive serological and molecular study to figure out the serostatus of HHV-6 among these immunocompromised patients. Of note, in Iraq, cancer has become a major public health issue and its burden continue to grow while our population is growing still without having embraced healthy lifestyles that may help to prevent many non-communicable diseases such as cancer as a results of decades of wars and occupation (Editorial, 2015). A Total of 20278 new cases of cancer were recorded in year 2011. 46.1% were male with incidence rate $55/10^5$ population and 53.9% were females with incidence rate $66.8/10^5$ population. The age range of patients was 10-70 years. Blood malignancies; leukemia, NHL and HL were ranked as third sixth and tenth of the commonest ten cancers by site in Iraq during 2011(Iraqi Ministry of health, 2014). Our results showed that (83.3%) of patients with AML and (61%) of healthy controls were positive for anti-HHV-6 IgG by IFAT, with a statistically significant difference between the two groups (P= 0.032). These results are totally consistent with results of Clark *et al*., 1990) who conducted a seroepidemiological case-control study by an indirect immunofluorescence assay and they reported a higher HHV-6 seroprevalence in acute myeloid leukaemia. On the other hand, our results are partially consistent with the results of a considerable serological study carried out by IFAT on patients with blood malignancies before chemotherapy; a group of AML has significantly higher HHV-6 seropositivity (Gentile *et al.*, 1999).

Concerning the results of ELISA technique, our results revealed a significantly higher anti-HHV-6 IgG positivity rate among patients compared to healthy individuals (p= 0.007). Our results are consistent with the previous results of (Salonen *et al*., 2002; Assem, *et al.*,2005), who reported a higher positivity rate of HHV-6 IgG among AML patients compared to control group. Although, in adult black South African patients with different types of cancers tested for herpesviruses 1-6, found two statistically significant associations: a decreasing risk of myeloid leukaemia and an increasing risk of oral cancer with increasing titers of antibodies against HHV-6 compared to all other patients (Berrington de Gonzalez, 2006)

The results also showed a significantly higher HHV-6 IgM among AML patients versus healthy controls (p= 0.0004). Basically, it is well known that detection of anti-HHV6 IgM refer to primary or more commonly reactivation infection in patient with AML or other immunocompromised patients (Fox *et al*,; 1990b; de Pagter *et al*., 2008). Our results are in agreement with the results of Salonen *et al*., (2002), who reported that leukemic children had significantly higher HHV6–IgM antibodies in comparison with reference subjects, and the antibody patterns in patients with ALL and patients with AML was not different. As the presence of anti-HHV6 IgM is a marker of active viral infection (Carrigan and Knox, 2000). Therefore, the high positivity rate of anti-HHV6 IgM in our patients may arise due to reactivation of preexisting virus, since the 77% of primary infection by HHV6 was occurred by the age 24 months, and that older siblings appears to be the source of HHV6 transmission (Zerr *et al*., 2005). The results of PCR found only one patient (3.3%) with AML was positive plasma viral DNA versus none in healthy controls. Similar low detection rate of HHV6 DNA was reported by Nefzi *et al*. (2012) who detected HHV6 DNA in 8% of blood samples and 4% in bone marrow samples of patients with AML. On the contrary, the HHV-6 DNA did not detect by use of blot hybridization in patients with AML (Josephs *et al*., 1988). Similarly, the Iraqi study was failed to detect HHV6 and HHV8 DNA among group of patients with AML (AL-Faisal and Al Baiyati, 2013). On another hand, two cases of monoblastic leukemia were described in which cancerous cells were found containing HHV6 component including genomic DNA, viral antigens in addition to particles of immature virus (Krueger *et al.*, 1994). More recently, using real-time quantitative PCR, a high percentage (26.8%) of chromosomally integrated HHV6 DNA was detected among ALL and AML patients in the Czech Republic (Hubacek *et al.*, 2009). Therefore, the question of a possible role of HHV6 in the oncogenesis of this type of malignancy is remaining controversial and further studies on large sample size is recommended. Our findings revealed that there was an increased infection rate of anti HHV-6 antibodies among patients with acute myeloid leukemia suggesting that the virus may have a role in the development of the disease.

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