

## Prevalence of Anti Human Herpes Virus-6 IgG and its Receptor in Acute Leukemia (Membrane Cofactor Protein: MCP, CD46)

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### ABSTRACT

**Background:** CD46 is a membrane cofactor protein, which acts as a cofactor for factor I proteolytic cleavage of C3, so it protects the cells expressing it on their surface from autologous complement attack. It has been recently described as a receptor for HHV-6. Also, it has been shown to be highly expressed on malignant cells as compared to normal cells, thus playing a major role by which these cells, either cells of haematological malignancy or cells of other body cancers, can protect themselves against complement attack so they can survive and metastasize.

**Patients and methods:** This study has been done to detect the seroprevalence of HHV-6 among 47 Egyptian adult cases of acute leukemia using the anti-HHV-6 IgG ELISA serological technique. CD46 receptor expression and immunophenotyping technique were performed using FCM. Twenty nine of the cases were ANLL, while 18 were ALL cases. Sixteen age- and sex-matched control cases were also studied for both anti-HHV-6 IgG and CD46 receptor expression.

**Results:** HHV-6 IgG antibodies were encountered in 29 (100%), 14 (77.8%) and 12 (75%) of the ANLL, ALL and the control group, cases, respectively. CD46 expression was encountered in 21 (72.4%) of the ANLL cases and in 10 (55.6%) of the ALL cases. Concordance between HHV-6 seropositivity and CD46 expression was encountered in 31 cases (29 positive and 2 negative). Discordance was encountered in 16 cases with 14 showing HHV-6 IgG seropositivity with no CD46 expression and 2 showing the reverse.

**Conclusion:** The lack of significant correlation between CD46 expression and seropositivity would exclude CD46 expression as a cause of contracting HHV-6 infection in leukemic patients.

**Key Words:** Anti-HHV-6 IgG - CD46 - ANLL - ALL.

### INTRODUCTION

Human herpes virus-6 (HHV-6) is a member of the roseolovirus genus of the B-herpetosub-family. It was first isolated from blood of six patients with lymphoproliferative disorders during investigations for new viral agents [1]. The virus differed from the previously characterized herpes viruses with respect to growth properties, antigenicity and genetic content. As the cellular and molecular biologic properties of independent isolates of HHV-6 were compared, isolates could be segregated into two groups, HHV-6 variants A and B [2]. It has been discovered that CD46 acts as an essential receptor for HHV-6 A and B variants [3]. Human CD46 is called Human Membrane Cofactor Protein (MCP). CD46 is a member of the complement regulatory protein family (RCA protein family). It is a membrane bound type-1 glycoprotein ubiquitously expressed on the surface of all nucleated human cells which acts as a cofactor for serum factor I serine protease cleavage of the C3b and C4b components of complements [4,5]. Cell surface expression of CD46 protects cells from lysis by autologous complement [4,6,7].

Leukaemia cells are usually exposed to blood, which contains complement. C3, which serves as a key effector of this complement system [8], is proteolytically activated and deposited as C3b on target cell membranes and then undergoes further proteolysis into fragments, namely C3bi and C3dg which are com-

petent to induce different biological reactions [8,9]. It is therefore important for tumor cells to circumvent C3 deposition if they are to escape complement-mediated cell damage, survive and metastasize [10-12]. It is accepted that human nucleated cells possess the C3-stepregulators, CR1, DAF and MCP, which partly explains the high resistance of nucleated cells to homologous complement attack [13]. As it is now realized that CD46 acts as a receptor for HHV-6 virus, we hypothesized that the expected high CD46 expression on leukemic cells might constitute a favourable environment for infection or reactivation of HHV-6.

Accordingly, we studied the expression of CD46 on malignant cells in ALL and ANLL patients as well as seroprevalence of HHV-6 virus among them to find if there is any possible correlation. We also correlated both parameters to standard hematological findings in ALL and ANLL.

#### **PATIENTS AND METHODS**

This work was performed on 47 newly diagnosed adults with acute leukemia, including 29 males and 18 females with an age range of 17 to 70 years. Twenty-nine of the cases were ANLL, including 19 males and 10 females. Their age range was from 20 to 67 years. The ALL cases were 18, of which 9 were males and 9 were females. The age range of the ALL cases was from 17 to 70 years. All patients presented to the Medical Oncology Department and the Clinical Pathology Department, National Cancer Institute (NCI), Cairo University. All patients were diagnosed according to standard methods including detailed history, thorough clinical examination, complete blood picture, bone marrow examination and cytochemical stains.

Immunophenotyping was carried out on mononuclear cells from peripheral blood or bone marrow using fluorescent labeled monoclonal antibodies (Mo Abs). Double marker labeling was performed, including proper isotype controls. The Mo Abs included: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, as T markers, CD19, CD20, CD22, CD24, cytoplasmic  $\mu$ , kappa and lambda as B markers, CD13, CD14, CD33 and myeloperoxidase as myeloid markers, CD16 & CD56 as natural killer markers and other markers as CD10, CD34 & HLA DR.

Phycoerythrin fluorescent labeled Mo Ab CD46 against human membrane cofactor protein (MCP) was included in the Mo Ab panel obtained from Serotec (UK). The reactivity was detected using XL-MCL flow cytometer (Coulter Corporation, Hialeah). A cut-off value of 20% expression was used to categorize the positivity for CD46. For detection of surface markers, the whole blood staining method was performed using Coulter Epics system (Coulter Corporation, Hialeah). In short, 10  $\mu$ l whole blood, incubated in the dark for 20 minutes, processed by the Q prep system where immunoprep reagent A for lysing, B as stabilizer and C as fixative were consecutively added. The samples were then analyzed on the flow cytometer and cases were assigned to different immunophenotypes as previously described [14]. Assay of serum HHV-6 IgG antibody was done using the Quantitative Diaclone ELISA Kit (solid phase sandwich Enzyme Linked Immunosorbent Assay). Sixteen healthy people of matched age and sex were included as control for serum HHV-6 IgG. At serum dilution of 1/20, negative samples were  $\leq 0.75$ , positive samples were  $\geq 1.00$  and equivocal samples were between 0.76-0.99.

Statistical analysis was done using the Chi square test for comparing independent qualitative data and Kruskal Wallis test to compare the two leukemia groups and the control group. The strength of agreement between CD46 expression and HHV-6 IgG was tested using Kappa measure of agreement.

#### **RESULTS**

This work was performed on 47 newly diagnosed adults with acute leukemia, including 29 males and 18 females with an age range of 17 to 70 years, a mean of  $35.06 \pm 13.49$  years and a median of 29 years. Twenty-nine of the cases were ANLL, including 19 males and 10 females. Their age range was from 20 to 67 years with a mean of  $35.28 \pm 12.58$  years and a median of 30 years. The ALL cases were 18, of which 9 were males and 9 were females. The age range of the ALL cases was from 17 to 70 years with a mean of  $33.86 \pm 14.37$  years and a median of 32 years.

HHV-6 IgG was positive at a dilution of 1/20 in all ANLL (100%), 14/18 ALL (77.81%)

and 12/16 (75%) healthy controls. The difference was statistically insignificant. The mean value of HHV-6 IgG in ALL leukemia was significantly higher than the control group ( $p = 0.02$ ). It was also higher in ANLL; the difference was nearly significant ( $p = 0.06$ ). No significant difference was encountered between ANLL and ALL (Table 1).

No significant association was encountered between the percentage of seropositive cases or the mean anti-HHV-6 IgG level on one side and the immunophenotype of ALL cases on the other side. No significant association was encountered between CD46 expression and either type of leukemia (ALL vs ANLL) in relation to the healthy controls. CD46 expression in positive cases is shown in table (2). It was 72.4% in ANLL, 55.6% in ALL. There was no significant association between CD46 expression and FAB subtypes in ANLL, or immunophenotype in ALL.

Fig. (1) represents the co-expression of CD46 and CD34 in ANLL, while Fig. (2) represents the co-expression of CD46 and CD19 in ALL.

No correlation was encountered between either anti-HHV-6 IgG level or CD46 expression on one side and either TLC or percentage blast on the other side. No correlation between anti-HHV-6 IgG level and CD46 expression was encountered in any of the tested groups.

Concordance between HHV-6 seropositivity and CD46 expression was encountered in 31 cases (29 positive and 2 negative). Discordance was encountered in 16 cases with 14 showing HHV-6 IgG seropositivity with no CD46 expression and 2 showing the reverse (Table 3). This was not in a good agreement, kappa = 0.074.

Table (1): Anti HHV-6 IgG serological level in acute leukemia cases with positive reactivity.

Group	No. of cases	No. of +ve cases (%)	IgG level (Eu/ml)		p-value
			Median	Range	
ANLL	29	29 (100)	4.65*	1.91-21.3	0.02*
ALL	18	14 (77.8)	5.35	1.08-11.03	
Control	16	12 (75)	3.45	1.02-4.89	

\* Significantly different from control  $p < 0.05$ .

Table (2): CD46 expression in the positive cases of acute leukemia.

Group	No. of +ve cases/ Total (%)	CD46 expression	
		Median	Range
ANLL	21/29 (72.4%)	28	22.20-90.00
ALL	10/18 (55.6%)	34.95	21.00-86.00
Control	0/16	3.05	0.6-11.3

Table (3): Concordance and discordance between HHV-6 IgG seropositive and CD46 expression in acute leukemias.

CD46	HHV-6 positive		HHV-6 negative		Total	
	No.	%	No.	%	No.	%
Negative	14	32.6	2	50	16	34
Positive	29	67.4	2	50	31	66

Kappa as a measure of agreement = 0.074.

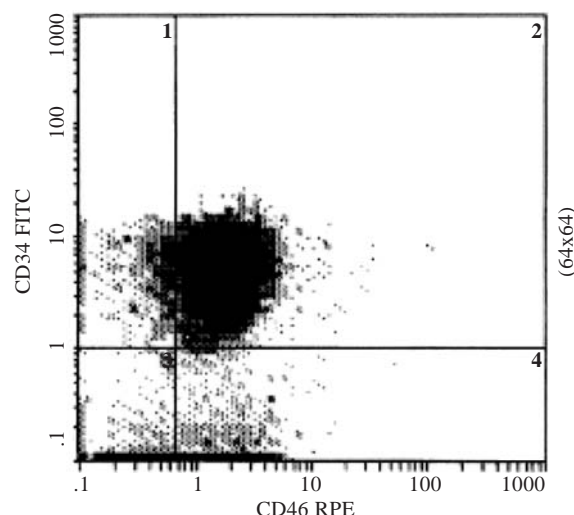


Fig. (1): Co-expression of CD34 and CD46 in an ANLL case.

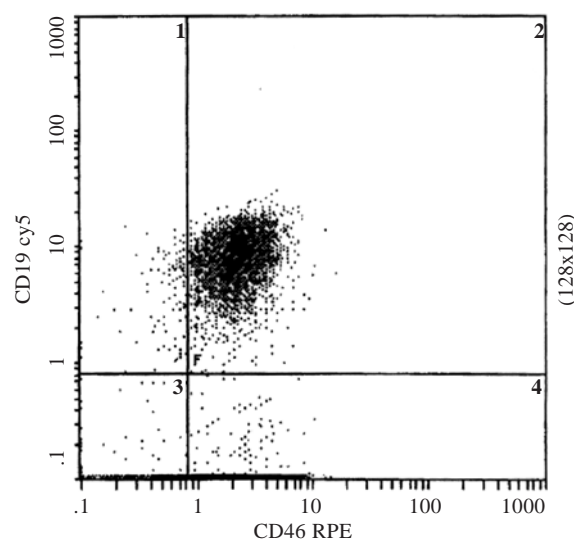


Fig. (2): Co-expression of CD46 and CD19 in an ALL case.

## DISCUSSION

Human Herpes Virus (HHV) is the etiologic agent of Exanthem subitum (ES) in children being responsible for more than 90% of cases, of fever in the first three years of life. Also, it causes opportunistic infections in immunocompromised patients and has been implicated in multiple sclerosis (MS) and in the progression of AIDS. Its reactivation has been reported as a cause of failure of hematopoiesis [3].

In this work, IgG anti HHV-6 was encountered in 100% and in 77.8% of ANLL and ALL patients, respectively, compared to 75% of the control. Clark et al. [15] reported that the prevalence of antibodies to HHV-6 in ANLL patients was 55%. In another large serological study conducted by indirect immunofluorescence (IFA) on patients with hematological malignancies who had not yet undergone immunosuppressive chemotherapy, a slight significant association could be reported between increased HHV-6 seropositivity and ANLL cases, with no association between this virus and ALL cases [16]. Levine et al. [17] also found no significant differences in antibody titers between 50 patients with ALL and 50 blood donors. On the other hand, Ablashi et al. [18] found higher levels of HHV-6 antibody in a small group of children with ALL compared to normal subjects.

In this work, HHV-6 seroprevalence in healthy adult persons has been encountered in 75%. The seroprevalence in healthy adult populations has been reported to be between 50 and 79% [19-22]. Recently, Pellett [23] showed that the seroprevalence of HHV-6 (A and B) variants was over 90% in healthy adults. Claudio and Steven [24], from an expert working group on HHV-6 and HHV-7 laboratory, denoted that HHV-6 seroprevalence using IFA showed a range of 39-100% among healthy adults and 80-100% among healthy children. The seroprevalence in this work was performed using the ELISA technique as it is a rapid, commercially available assay for the detection of IgG antibody to HHV-6. Theo and coworkers [25] denoted that the results obtained by the ELISA method were in close agreement with the immunofluorescent assay as it showed a sensitivity of 99.7% and a specificity of 98.75%; so, it is considered as a useful aid in the diagnosis of HHV-6. Also, ELISA is a sensitive and reliable alternative to IFA without the problem of non-specific reac-

tivity often found with IFA [26], which may be the cause of the high percentage of positive cases in the studies performed using this latter technique.

In this work, the mean of HHV-6 IgG serological level was statistically significantly higher ( $p < 0.02$ ) in the group of ANLL patients compared to healthy controls and was nearly significant ( $p < 0.06$ ) in the group of ALL patients compared to the group of healthy controls. The association between increased HHV-6 seropositivity and AL cases was reported by Gentile et al. [16]. As regards HHV-6 IgG seropositivity among the immunological subtypes of the lymphoid cases, no significant difference was observed regarding the number of positive cases. The number of cases, however, is too small to show any potential difference.

In this work, the percentage of blast cells expressing CD46, the recently known viral receptor for HHV-6, in the three studied groups (ANLL, ALL and control) showed that CD46 in ANLL patients was 72.4%, in ALL patients was 55.6% and 0% of the control adults, which indicate high level of CD46 expression in myeloid cases, followed by lymphoid cases. However, this difference was not statistically significant. This finding is in agreement with Hara et al. [27] who reported that CD46 expression, by using FCM, was increased in the majority of haematological malignancies. Also, its levels of expression on T and myeloid cell lines were usually 2-8 folds higher than those on their normal counterparts. This also was in close agreement with a study done by Tomoko et al. [28] who studied CD46 (MCP) in different hematological diseases and detected that CD46 expression was increased in most CML, CLL and in the majority of ANLL, ALL and NHL cases and that the high level of MCP reflects some malignant transformation or an immature stage in blood cells.

Different authors [10-12] considered the presence of CD46 on the cell as a protective mechanism for the cell itself against complement-mediated lysis. The high expression of CD46 on blast cells is important for these cells to circumvent C3 deposition if they are to escape complement-mediated cell damage and survive and metastasize. Hourcade et al. [13] partly explained the high resistance of nucleated cells to homologous complement attack by the finding

that human nucleated cells possessed the C3-step regulators including the MCP.

In this work, concordance between HHV-6 seropositivity and CD46 expression was encountered in 31 cases (66%) (29 positive and 2 negative). Although CD46 was discovered to act as an essential receptor for HHV-6 A and -6B variants, discordance was encountered in 16 cases (34%) with 14 showing HHV-6 IgG seropositivity and negative CD46 and 2 showing the reverse.

A possible explanation for HHV-6 IgG seropositivity with negative CD46 may be taken from the work of Montgomery et al. [29] who demonstrated that CD46 was selectively and progressively down regulated from the target cell surface during the course of HHV-6 infection, i.e. after infection down regulation of receptor expression took place. On the other hand, the reverse could be explained by the work of Santoro et al. [3] who demonstrated that CD46 as a receptor was not sufficient to enable HHV-6 fusion/entry, since some human T cell lines were found to be non permissive for HHV-6-mediated fusion and replication despite the presence of significant surface CD46 levels. Hence, HHV-6-mediated fusion requires an additional factor besides CD46, possibly a viral co-receptor, that is not uniformly present in all human cell types, which is well documented for other enveloped viruses such as HIV-1 [30] and HSV-1 [31].

CD46 as a receptor for HHV-6 and its down modulation by this viral infection [3] can be very helpful in the damage and clearance of the infected cells including the cancer cells. The down modulation of this complement regulatory protein "CD46" secondary to HHV-6 infection might induce complement activation and contribute to occurrence of virus-induced apoptosis of the malignant cells [32].

A central question which arises now is: can infecting cancer cells, including hematopoietic blast cells expressing high levels of CD46 on their surface with HHV-6 which uses CD46 as a receptor, help in the damage of these unwanted cells and controlling its proliferation? However, the effect of infection itself on immunocompromised patients should be taken in consideration.

In conclusion seroprevalence of anti HHV-6 in ALL and ANLL is comparable to the control group, though positive ANLL cases show significantly higher titre. The lack of significant correlation between CD46 expression and seropositivity would exclude CD46 expression as a cause of contracting HHV-6 infection in leukemic patients. The therapeutic potential of HHV-6 infection needs further studies.

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