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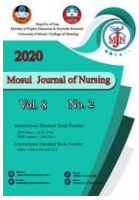

Seroprevalence of EBV antibodies in children with acute lymphoblastic leukemia

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Hawry AliHaedar Hussein Zangana[1] Zakarea Abdullah Yaseen Alkhayat[2]

Abstract

Background: *Epstein Barr virus* (*EBV*) has a unique association with several human malignancies, especially lymphoproliferative disorders.

Objective: The aim of the present study was to estimate the levels of EBV serum antibodies in children with ALL.

Material and Methods: Thirty-one children with acute lymphoblastic leukemia who were attending Nanakali oncology -hematology department in Erbil City. were subjected for antibody detection of Epstein-Barr (EBV) virus with age ranged (2-12) years. A second group comprised of 30 children with non-malignant condition age ranged (2-12) years regarded as control group.

Both groups were tested for Epstein-Barr virus antibodies assays (EBV-VCA (IgG, IgM), EBV-EA (IgG, and IgM) by enzyme -linked immunosorbent assay (ELISA).

Results: Regarding the patient groups (ALL) twenty-five (25;80.7%) patients had raised antibody levels while in the control healthy group, thirteen (13;43.3%) child had raised antibody levels. Regarding the patients group the numbers and percentages of VCA-IgM (4;12.9%), VCA IgG (12;38.7%), EA IgM (3;9.8%), and EA IgG (6;19.4%) were higher when compared with the control group. The mean values of

1 Hawler Medical University, College of Medicine, Dept. of Basic science e-mail : hawry_zangana@yahoo.com tel : 07504383339 Erbil- Iraq
2 Hawler Medical University, College of Medicine, Dept. of Basic science e-mail : zakarea.abdullah@hmu.edu.krd tel : 0750 486 8609 Erbil – Iraq

EBV (VCA IgG) in ALL group increase in a highly significant manner ($p \leq 0.01$) when compared with the control group, While no significant difference between ALL and control in the mean values of VCA IgM. There was a significant increase in the mean of EA IgG in ALL than in control group ($p \leq 0.05$), whereas, no significant differences were noticed in EA IgM between the two groups.

Conclusion: The current study shows evidence of active EBV replication in a significant number of Acute ALL patients.

Keywords: Acute lymphoblastic leukemia (ALL), Epstein - Barr virus (EBV), early antigen (EA), viral capsid antigen (VCA).

Introduction:

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and represents around 23% of cancer diagnosis below 15 years of age, amounting to an annual incidence of approximately 30-40 per million cases (Alibek et al., 2013). In the United State, there are About 2,200 cases of childhood leukemia (ages <14 years) are recorded annually; 79% of these cases are acute lymphoblastic leukemia (ALL), followed by acute myeloblastic leukemia (AML), chronic myeloid leukemia (CML), and other types (Greaves, 2006). There has been a gradual increase in the incidence of ALL in the past 25 years with a peak incidence in children aged 2 to 3 years (Khaleel, 2018). Viruses are linked with cancer emergence in about 15% of malignancies (Alibek et al., 2013). Epstein-Barr Virus (EBV) is a member of the Herpesviridae family. EBV infections exist worldwide. Generally, these infections emerge in early childhood (Alibek et al., 2013; Keresztes et al., 2006). EBV may have an important role in some hematologic malignancies due to their capability to modify the host's immune system (Greaves, 2006). It is now believed etiologically connected with the endemic Burkitt's lymphoma, Hodgkin disease, and nasopharyngeal carcinoma (MacKenzie et al., 2006). Former studies demonstrated that EBV intervenes with cellular DNA renovation mechanisms and could procure to genetic changes in the infected cells (Tedeschi et al., 2007). The concurrence of EBV infection and acute leukemia has been scarcely announced (Sehgal et al., 2010; Tedeschi et al., 2007). The present study aimed to estimate the levels of EBV serum antibodies in children with ALL.

Material and method:

Study protocol

This was a controlled case study which was performed from the period between January 2019 and January 2020. The study population included children attending the Nanakali center, hematologic oncology department in Erbil.

Ethical considerations

This study was approved by the Ethics Committee of Hawler Medical University, Erbil. The parental endorsement in both written and

oral forms was acquired for their children to be embroiled in the study. Before the blood sample was collected, the procedure was thoroughly explained to every person to ensure that they understood exactly what was going to happen. It was also pointed out to the individuals that they could refuse to participate in the study without prejudice.

Study population

The present study was achieved on patients with acute lymphoblastic leukemia (ALL). Thirty-one (31) children newly diagnosed with ALL were involved in this study. The diagnosis is made by bone marrow examination and the slides reviewed by two hematologists. The age of leukemic children range (2-12) years 19 males and 12 females. Patients on treatment were excluded from this study. The control group included thirty (30) looking healthy children with age ranged (2-12) years which were not complaining of any complaints.

Analytical methods.

Venous blood was withdrawn (2ml of blood) from the ALL group and the control group put in clot activator tubes. The following tests were performed: viral capsid antigen (VCA-IgG-VCA-IgM) and early antigen (EA-IgG, EA-IgM) using enzyme-linked immunosorbent assay (ELISA). The procedures were done according to manual instruction of the ELISA kit of (IMMUNOLAB GmbH), Purified EBV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, the anti- EBV specific antibody, if present, will bind to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls. The final measurement is carried out at 450 nm. Then Cut-off OD value was obtained according to the instructions. Then the calculation of the Index of each determination was achieved by dividing the OD values of each sample by the obtained OD value of Cut off as explained by the manufacturer.

Interpretations

According to the manufacturer instructions, the Index Values or OD ratios are interpreted as follows:

Negative	≤ 0.90
Equivocal	0.91 to 1.09
Positive	≥1.10

Statistical Analysis

The data analysis was accomplished through utilizing descriptive statistics, including mean ± standard deviation, frequency, and frequency percentage. Comparisons were made using Student's *t*-test by employing standard equations. The results were announced with $p \leq$

0.05 or $p \leq 0.01$ as the accepted level of significance accordingly.

Results:

Table (1) illustrates the distribution of positive cases of EBV antibodies in both patients and control. It is noted that in the patients' group (ALL) that the numbers and percentages of VCA-IgM (4;11.8%), VCA IgG (18; 58.1%), EA IgM (3; 9.7%), and EA IgG (16;51.6%) were higher when compared with the controls group.

Table (1) Distribution of positive cases of EBV antibodies in ALL and control groups

Groups	VCA- IgM		VCA-IgG		EA-IgM		EA-IgG		Total	
	N	%	N	%	N	%	N	%	N	%
Patients (ALL)	4	12.9	12	38.7	3	9.7	6	19.4	25	80.7
Control	2	6.7	8	26.7	1	3.3	2	6.7	13	43.3

Table (2) demonstrates a comparison between the mean levels of index values of EBV antibodies in the patients and control groups. The VCA IgG mean values of EBV in ALL groups showed a highly significant increase ($P \leq 0.01$) when compared with the control group, while no significant difference ($P>0.05$) between ALL and control in the mean values of VCA IgM. The mean of EA IgG was significantly higher ($P \leq 0.05$) in ALL than in the control group, while, no significant ($P>0.05$) differences were noticed in EA IgM between the two groups.

Table (2) Comparison between mean levels ± SD of index values of EBV antibodies in the ALL and control groups.

EBV antibodies	ALL (31)	Control (30)	Statistical analysis (t-test)
VCA-IgM	0.71 ± 0.23	0.63± 0.41	t = 0.9439 df = 59 NS*
VCA-IgG	1.83 ± 0.92	1.01 ± 0.74	t = 3.8281 df = 59 HS*** (P≤0.01)
EA-IgM	0.53 ± 0.72	0.61 ± 0.43	t = 0.5247 df = 59 NS*

EA-IgG	1.2 ± 0.51	0.89 ± 0.47	t = 2.4665 df =59 S** ($p \leq 0.05$)

*** NS: Non-Significant, ** S: Significant, *** HS: Highly Significant**

Discussion:

The present study delineates that the prevalence of EBV antibodies was higher among children with acute lymphoblastic leukemia. Many literatures point toward the controversial role of EBV in childhood leukemia (Sehgal et al., 2010). The coinfection with EBV has also been linked with Hodgkin's disease, large cell lymphoma, acquired immunodeficiency syndrome (AIDS) related lymphoma, and possibly chronic lymphocytic leukemia in adults (Keresztes et al., 2006). There are very few records in world literature on the causal role of EBV in childhood leukemia (Khan, 2010). EBV infection in patients with lymphoid leukemia may be a factor involved in the high incidence of pediatric leukemia in Sudan (Ahmed et al., 2012). (Schlehofer et al., 1996) from Germany achieved EBV serology in 121 children with acute lymphocytic leukemia (ALL) and announced an increase in antiviral capsid antibodies (VCA) in these children. In a prospective study (Lehtinen et al., 2003) concluded about the linkage between the elevated levels of antibodies to EBV-early antigen (EA) and Epstein-Barr nuclear antigen (EBNA) and the increased risk of lymphoma/leukemia. (Loutfy et al., 2006) has also studied antibody for EBV in children with leukemia. They observed that EBV was positive in 83% of leukemic children and 95% of the control subjects were also positive for EBV.

The relationship of EBV with childhood leukemia, mainly lymphoblastic leukemia (ALL), has been found in some seropositivity studies, genetic analyses, and epidemiological studies (Altieri et al., 2006). Firstly, it was noted that EBV viral capsid antigen (VCA) IgM, in EBV-seropositive mothers, was associated with increased risk of acute ALL in offspring (O'Connor & Boneva, 2007). The presence of VCA antibodies is recognized as a sensitive measurement for active infection. While for EBV reactivation, testing for EBV early antigen-antibody was found to be useful (Khan, 2010; Sehgal et al., 2010). There are several mechanisms by which EBV could increase the risk of malignant transformation of infected cells. It was found that viral proteins inhibit apoptosis, affect the JAK/STAT pathway, promote epigenetic changes, and undermine the immune defense mechanisms (Clemens, 2006; Raab-Traub, 2012). Children with latent infection seemed to be at higher risk of ALL as compared to children with acute infection (MacKenzie et al., 2006). Following a primary infection in healthy individuals, EBV infects and immortalizes B lymphocytes, which is followed

by a lifelong viral latency (Schlehofer et al., 1996). The proliferation of EBV infected B-cells is restrained and monitored by an adequate T-cell dependent specific immune response. However, a strong reduction in the numbers of EBV-specific T lymphocytes may result in reactivation of the virus and, eventually, the development of lymphoproliferative disease (MacKenzie et al., 2006; Tedeschi et al., 2007). The EBV BZLF1-encoded replication activator (ZEBRA) is a key mediator of reactivation from latency to the viral productive cycle. Among the EBV transactivators, the ZEBRA protein plays a pivotal role in transforming the virus from a latent to a productive mode (Germini et al., 2020; Raab-Traub, 2012). Utilizing sensitive and specific molecular tools like Real-time PCR assay, EBV LMP1 gene transcriptional activity was noted in a considerable proportion of patients with acute lymphoblastic leukemia (El-Sharkawy et al., 2018). (Ahmed et al., 2012) had observed that EBV LMP1 gene transcriptional activity was in a large proportion of Sudanese patients with acute lymphoblastic leukemia. They suggested that EBV may be a factor involved in the high incidence of pediatric leukemia in the Sudan. On the other hand, (Lehtinen et al., 2003) from National Public Health Institute, Oulu, Finland had identified a link between maternal EBV reactivation and the development of ALL in offspring, suggesting an association between maternal EBV infection and risk of Leukemia in the offspring. EBV infection might play a role in the progression of leukemia and might be a useful indicator of worsening the clinical course of the disease (Loutfy et al., 2006). The limitation of the present study was the small sample size which limited of the comparison of the variables in subgroups. Another limitation of the current study was the seropositivity of EBV which is considered as only a marker of latent infection and does not necessarily mean the reactivation of latent EBV infection.

Conclusion:

The present study suggests that a significant number of patients with ALL show evidence of active EBV replication. The present suggest [that infection with EBV may be a triggering factor for ALL development.

Recommendations:

PCR testing is mandatory to explain the linkage of EBV associated with childhood ALL.

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Conflicts of interest:

There are no conflicts of interest.

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