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IMMUNOLOGICAL DETECTION OF EPSTEIN-BARR VIRUS IN IRAQI PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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ABSTRACT
Epstein-Barr virus (EBV) is a gamma herpes virus which infects the epithelial cells and B lymphocytes. The EBV considered an important etiologic in various tumors factor. This subject has been attracted much attention in the recent years but a little is known about its relation with leukemia. There are several controversial hypothesis proposed to identify the responsibility of the physical, chemical and even biological factors in the Leukemia incidence. EBV is able to cause a lytic and latent infection of B-lymphocyte and epithelial cells. It might cause many cancerous diseases. The aim of study is to detect the antibody concentration of IgG against the viral antigen EBNA1 in the Iraqi Arab patients with chronic lymphocytic leukemia by using Enzyme immunoassay (ELISA). The study included 55 patients with chronic lymphocytic leukemia (before and after treatment with flodarabine) comparing with 30 healthy individuals as a control group. The results revealed that the total count of white blood cells (WBC) was significantly high ($P<0.01$) in patients as compared with controls as well as the patients before treatment compared with patients after treatment. According to the ELISA, results showed that the differences between patients and healthy subject were not significant whereas the differences between the patients before and after treatment were significant ($P<0.01$).

KEYWORDS: Epstein-Bar Virus (EBV), Leukemia, IgG, white blood cell.

INTRODUCTION

Leukemia is defined as a series of malignant disorders characterized by increasing the abnormal white blood cells in the bone marrow and blood stream[1,2]. These cancer cells are attributed to non-programmed death[3]. The rate of leukemia in Iraq increased with the increasing of the cancer cases. It is the third most common cancer in Iraq for 2012[4]. As a disease Leukemia is divided into acute and chronic by severity of the disease and is divided into lymphocytes and Myeloid depending on the type of infected cells [5]. Chronic lymphocytic leukemia is a type of lymphatic proliferation disorder; this type is characterized by the accumulation of lymphocytes of small size than normal and nonfuel maturity in the bone marrow, blood stream, lymph nodes, spleen. Chronic Lymphocytic leukemia affects older people, with the highest incidence among ages (60-80) years. A symptom of the disease includes; inflation of lymph nodes, liver, spleen, acute anemia, bleeding, bruising, and feeling tired and fever [6]. Chronic lymphocytic leukemia occurs for several reasons, including genetic mutations, chromosomal mutations, exposure to radiation, chemicals and infection with viruses [2, 12]. One of the most important viruses that have been shown to be related to this disease is Epstein Barr virus (EBV) which is classified in the family Herpesviridae subfamily Gamma herpes virinae that infects more than 90% of population [7]. The virus consists of a protein heart wrapped with DNA. It is surrounded by a nuclear portfolio. The diameter of the mature virus (120-180) nm. The virus genome encodes to more than 80 viral protein [8]. EBV invades and replicates in epithelial cells of the pharynx causing the primary infection which is mostly asymptomatic during childhood. Some patients develop various severity of fever, sore throat, swollen lymph glands and occasionally swelling of the spleen[9]. The virus transfer by saliva exchange[10] then EBV spread into lymphocytes specially to B cells and persevere a long life in it [11]. The EBV considered one of tumor viruses as it related with several cancers like Burkitt`s lymphoma, nasopharyngeal carcinoma, gastric carcinoma, Hodgkin`s and non-Hodgkin`s lymphoma, post-transplant lymphoproliferative disease and X-linked lympho proliferative disease [12 ,13]. The aim of the present study is to detect antibody concentration of IgG against the viral antigen EBNA1 in Iraqi Arab patients with chronic lymphocytic leukemia by Enzyme immunoassay (ELISA) as compared with healthy patients.

MATERIALS & METHODS

The study includes 85 individuals. They were divided into two groups, patients group, included (55) were been (31 males, 24 females), before and after treatment (before treatment 24 patients (8) female and (16) male, after treatment (31) patient (15) female and (16) male), and healthy group included 30 (15 males, 15 females). Peripheral blood samples were collected as a (3ml) in EDTA tubes for both patients and controls individuals in the study. Wight Blood Cells count (WBC) was calculated by hematology analyzer (hemolyzer 5/anlyticon Germany). Immune testing was performed for patients to detect IgG antibodies before and after treatment. The results were compared with the healthy control group. The

test was performed using immunosorbent bonding technique (ELISA) and using EBV IgG EBNA1 kit (Demeditec, Gremany) according to the company's supplied instructions.

Statistical analysis

SPSS version 16 and Excel software were used to analyze the results. Means were compared by using t –test. P<0.05 is considered significant.

RESULTS & DISCUSSION

In this study the total count of white blood cell (WBC) was calculated. The results shown in table (1) exhibited high significant differences (P<0.01) in the WBC between patients and control as it was (26.879±5.36), (7.260 ± 0.215) respectively.

TABLE 1: The total count of white blood cells in the patients and control

Study group	Mean ± SE		p-value
	Patients	Control	
WBC $x10^3$/ µL	26.879 ± 5.36	7.260 ± 0.215	<0.01

These results are consistent with a local study on acute myeloid leukemia which showed there were significant differences in the number of WBC in both the patients and control (Tuama, 2016)[14]. The results of the study, as shown in Table (2) non-significant differences in patients in terms of sex between males and females as it was (33.7 ± 8.89) for males, (18.17 ±4.27) for female.

TABLE 2: The total count of the white blood cells for patients by sex

Study group	Mean ± SE		P-value
	Female	Male	
WBC $x10^3$/ µL for Patients	33.70 ± 8.89	18.17 ± 4.27	0.1 NS

The results of total count of the WBC showed no significant differences between males and females. These results are similar to the results obtained by Tuama, 2016 study[14] who founds that there were no significant differences in the number of the WBC between male and female with acute myeloid leukemia.

The statistical analysis of the total count of the WBC in Table (3) showed that the mean of the number of the WBC is significantly increased (P<0.01) in patients before treatment (50.37 ±11.96) compared with the patients after treatment (11.63± 1.81). The decrease of total number of WBC after treatment is related to medication used to treat patients with chronic lymphocytic leukemia (flodarabine). It is a chemical treatment inhibits the activity of the cell by inhibition of enzymes involved in DNA replication and RNA synthesis [15].

TABLE 3: Total count of white blood cells in the patients before and after treatment

Study group	Mean ± SE		p-value
	before treatment	after treatment	
WBC x103/ µL for Patients	50.372 ± 11.96	1.81 ± 11.636	<0.01

Table (4) showed significant differences (P<0.05) in the total number of the WBC in female patients before treatment (34.716 ±12.1) as compared with the female patients after treatment (4.69 ±0.51). Also the results showed significant differences (P< 0.01) in the total number of the WBC of the male patients before treatment (58.20 ±16.81) as compared with the male patients after treatment (12.08 ±3.08).

TABLE 4: The number of white blood cells in female and male patients before and after treatment

Study group	Mean ± SE		p-value
	Before treatment	After treatment	
WBC $x10^3$/ µL female patients	34.716 ± 12.1	4.69 ± 0.51	<0.05
WBC $x10^3$/ µL male patients	58.20±16.81	12.08± 3.08	<0.01

The results could be attributed to the using of treatment as the mechanism of action of flodarabine depends on destruction of lymphocytes therefore, the number of WBC decreased after treatment. These results agreed with another study conducted on patients before and after the treatment which was used (Rituximab) to treat the high number of B lymphocytes in non- hodgkin's lymphoma, chronic lymphocytic leukemia, and autoimmune diseases [16].

Immunohistochemistry (results of enzymatic association with immunosuppression)

Results of the current study showed that the means of the enzymatic binding of the antibody IgG EBNA1 (Table 5) was not significant in the patients (7.82 ±0.46) as compared with the control (8.09 ± 0.40) .

TABLE 5: The enzymatic binding of IgG EBNA1 between patients and control

Study group	Mean ± SE		p-value
	Patients	Control	
EBV IgG EBNA1	7.82 ± 0.46	8.09 ± 0.40	0.6 NS

The results are consistent with the results obtained by Al-Hashemi (2014) which was conducted on Iraqi patients with non- hodgkin's lymphoma to detect the Epstein Barr Virus in their serum[17]. Also the results agreed with another study which showed no significant differences between patients and control as all healthy adult individuals carry the Epstein Barr virus through the response to nuclear antigen of the virus (EBNA1 IgG) [18].

The table (6) showed the mean of the enzymatic association of IgG EBNA1 antibody. The differences between male patients (6.96 ± 0.66) were not significant.

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TABLE 6: The IgG EBNA1 antibody for male and female patients

Study group	Mean ± SE		P-value
	Male patients	Female patients	
EBV IgG EBNA1	8.63 ± 0.74	6.96 ± 0.66	0.1 NS

Results showed the IgG EBNA1 antibody concentration (Table 7) was significantly higher ($P<0.01$) in the patients before treatment for both sexes (10.20 ±1.06) than the patients after treatment for both sexes (7.51 ± 0.55).

TABLE 7: The EBNA1 IgG antibody concentration for patients before and after treatment

Study group	Mean ±SE		P-value
	Before treatment	After treatment	
EBV LgG EBNA1	10.20 ± 1.06	7.51 ± 0.55	0.03

These results are consistent with the results of another study on patients with autoimmune disease which showed that there was a significant difference ($p\leq 0.05$) in EBNA1 IgG between patients before treatment and patients after treatment [16].

Results of the current study showed that 1% of patients were negative to the antibody EBV EBNA1 IgG. This result is consistent with other studies conducted in several communities (Spain, France, Germany and Italy) [18]. According to Al-Hashemi, 2014), the proportion of people who carry the virus with IgG antibodies in serum ranged 98 – 99 % [17].

The current study showed that 100% of healthy people (control) have EBV EBNA1 IgG. The spread of this virus could occur as a result of the virus transmitting among the children in schools or by the sharing brothers bedding or by saliva. Moreover, it is called kissing disease because of the ease of its spread[9]. There are no symptoms in the children, while the appearance of symptoms could be detect in the adolescence or adulthood. The infection remains latent and the continued presence of antibodies in the serum for many years and memory cells are active for many years [4].

We concluded that the appearance of the antibody EBNA1 IgG in 99% of patients and 100% of healthy (control) were equal. It means there is no association with the occurrence of chronic lymphocytic leukemia. However, the concentration of antibodies in patients, especially before treatment, can be increased due to an increasing in the number of B lymphocytes.

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