Epstein- Barr Virus In Iraqi Patients With Nasopharangeal Carcinoma

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ABSTRACT:

BACKGROUND:

Epstein-Barr virus (EBV) was ubiquitous Herpes virus that had a role in the development of undifferentiated carcinoma of the nasopharynx, Burkett's lymphoma, acute infectious mononucleosis and other lymphoprolifrative disorders.

METHODS:

Thirty Iraqi patients with nasopharangeal carcinoma were referred to Oncology Unit in Al-Kadhemia Teaching Hospital from 1992-1994. Sera of those patients were tested for the presence of antibodies against Epstein-Barr virus nuclear and early antigens using indirect immunoflourescence test. Cellular immunity for those patients was tested for the CD4+, CD8+, CD4/CD8 ratio, T-cells % and B -cells %. Their results were compared with twenty-two normal apparently normal individuals.

RESULTS:

Antibodies to Epstein-Barr virus nuclear and early antigens were detected in nasopharangeal carcinoma Iraqi patients and not in the control group. There was significant difference between two groups in CD8+ cells, T- cells % and B- cells % and there was no significant differences between two groups in CD4+ cells, CD4/CD8 ratio.

DISCUSSION:

EBV infection was stopped by T- cells immune response that was capable of eliminating virus infected cells and virus neutralizing antibodies against nuclear and early antigens which prevent the spread of infection. Lymphocytes were predominantly CD8+ cytotoxic T lymphocytes, which recognize and destroy EBV infected cells.

RECOMMENDATIONS:

Other antibodies to viral capsid antigens (IgG, IgA and IgM). Other methods must be used other than indirect immunoflourescense test like western blot method and enzyme linked immune sorbent assay (ELISA).

KEY WORDS: Epstein-Barr virus, nasopharangeal carcinoma, antibodies.

INTRODUCTION:

Cancer can be induced by different causes, one of them viral infection. The best example was undifferentiated carcinoma of the nasopharynx that Epstein-Barr virus (EBV) had a role in the development of this tumor (2), this virus is a member of the Herpes group of viruses. By the age of three years 99% of children in developing countries had been subclinicaly infected with EBV while in developed countries, infection occurred between the 15-25 years (1).

The virus was excreted in oropharangeal secretions and responsible for person-to-person transmission. This virus was isolated from cells of an east African individual with Burkitt's lymphoma. In fact, Epstein and Barr discovered this in 1964.

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It was also associated with nasopharangeal carcinoma by replication of this virus in the epithelial cells of some nasopharangeal carcinoma, a tumor that occurred primarily in China, and with thymic carcinoma and B-cells lymphoma in the United States. However, cells from Burkitt's lymphoma patients in the United States show no evidence of EBV infection while cells isolated from East African individuals with Burkitt's lymphoma contain EBV DNA and nuclear antigens (6,7). Viral EBV antigens were divided into three classes based on the phase of the viral life cycle in which they were expressed: latent phase antigens, these included EB nuclear antigens and LMP1 antigens. Their expression revealed that an EBV genome was present and revealed past infection. Second antigens were early antigens, which included non-structural early antigens; their expressions indicated the onset of productive viral replication and were often found in nasopharangeal carcinoma.

Lastly, late antigens that included viral capsid and viral envelope antigens. They were produced abundantly in cells undergoing productive viral infection (7). Immunity to (EBV) infections elicited an intense immune responses consisting of antibodies against many virus specific antigens, a number of cell mediated responses and secretion of lymphokines (7). Primary EBV infection was stopped by two defenses: cellular immunity by T – cells immune responses capable of eliminating almost all virus infected cells with characteristic atypical lymphocytes were predominantly CD8+ cytotoxic T lymphocytes. The other arm of immune responses was humoral immunity by induction virus neutralizing antibodies which prevent the spread of infection from one target cells to other and the pattern of antibodies responses to different EBV antigens help in distinguishing acute or subclinical infection from past EBV infection (1,3,17).

EBV escaped from immune system and cause viral persistence by down regulating the expression of highly immunogenic antigens of the virus or by direct modulation of the host cytotoxic T lymphocytes responses by virus encoded proteins (3). Immunological and virus serological testing were frequently performed in order to determined the pattern of specific antibodies to different classes of EBV antigens and their diagnostic and prognostic significance.

The aim of this study was to detect whether those patients had a high level of antibodies against EBV, and to study the cellular immune responses in Iraqi patients with nasopharangeal carcinoma caused by EBV infection. So this revise mainly concerned EBV serology and related immunological parameters.

PATIENTS AND METHODS:

1. **Patients group:** consisted from thirty Iraqi patients with nasopharangeal carcinoma who were referred to Oncology Unit in Al-kadhemia Teaching Hospital from 1992-1994. Their age ranged from 25-65 years. Males were fifteens and the rest was females.

2. **Control group:** included twenty-two apparently healthy individuals. Their age ranged from 23-66 years. Males were thirteen and the rest was females.

METHODS:

1. Sera from both groups were separated from the blood and tested for the presence of antibodies directed against EBV nuclear antigens and early antigens using indirect immunofluorescence test (GULL LABORATORIES, USA).

2. Lymphocytes from the blood of both groups were separated using Ficol-Hypaque lymphocytes separation media and then enumeration of lymphocytes subsets (CD4+, CD8+, CD4+/CD8+ ratio) were done using indirect immunofluorescence test (16).

3. Counting of T-lymphocytes was done using sheep rossating method and enumerations of B-cells were done by direct immunoflourescence test (16).

Statistical analysis was done using student t-test.

RESULTS:

Serological test for detecting antibodies against EBV antigens (EBVNA) were detected in 93.33% of the patients with nasopharangeal carcinoma living in different parts of Iraq.

Antibodies against EBVEA were also detected in 93.33% of patients. There was a significant differences (P>0.05) between patients group and control group in these two antibodies as shown in table-1-. In case of testing lymphocytes subsets (CD4+, CD8+ and CD4+/CD8+), there was a significant difference (P>0.05) between two groups in cytotoxic T-cells CD8+ cells only. The mean of this cells was 27.56 while in the control group was 20.40 as demonstrated in table-2-.

The last table –3- showed the significant differences (P>0.05) in the percentages of T and B-cells between these groups. In the patients group the percentage of T-cells was 37.1% and B-cells was 3.33%.

Table1: Antibodies to EBV nuclear antigens and to EBV early antigens in Iraqi patients with nasopharangeal carcinoma compared with control group.

Serological tests	Patients group(♂ and♀) Positive reaction No.= 30		Control group Positive reaction No.=22	
	No.	%	No.	%
EBVNA	28	93.33 (1)	1	4.54
EBVEA	28	93.33 (1)	1	4.54

(1) P>0.05

Table 2: Lymphocytes subset in Iraqi patients with nasopharangeal carcinoma (mean +_ standard error mean)(X+_ SEM) and control group.

Lymphocytes subsets	Patients group (♂ and♀) No.=30	Control group No.=22
CD4+% X+- SEM	38.16 +_ 4.03 (N.S.)	41.22+_3.03
CD8+ % X +- SEM	27.56+_2.02 (1)	20.40+_1.03
CD4+/CD8+ ratio X+- SEM	1.53+_0.21 (N.S.)	1.95+_0.35

(1): P>0.05
(N.S.): Not significant.

Table3: Percentages of T and B-lymphocytes of Iraqi patients with nasopharangeal carcinoma in comparison with control group.

Tests	Patients group (♂ and♀) No. =30	Control group No.=22
T cells % X+_ SEM	37.10 +_ 1.76 (1)	50.90+_ 2.86
B cells % X+_ SEM	3.33 +_ 0.25 (1)	5.18+_ 0.36

(1): P>0.05

DISCUSSION:

Epstein –Barr virus was a member of human herpes virus family and like other herpes virus maintains a life long latent association with B-lymphocytes and a permission association with stratified epithelium in the oropharynx. Clinical manifestations of primary EBV infection range from acute infectious mononucleosis to symptomatic persistence infections. EBV was also associated with a number of malignancies in the human by induction B-cells proliferation and activation of a cellular oncogens (4,6,7).

The studying of immune responses in EBV infected patients was important because some patients with EBV infections had failure of immunity and induction of malignancy in patients receiving immune suppressive therapy (1). In the present study, several standard immunological and serological parameters were tested. We found that sera from Iraqi patients with nasopharangeal carcinoma contained elevated levels of antibodies to different viral specific antigens {EBV nuclear (EBNA) 93.33 % and EBV early antigens (EBEA) 93.33 %}, which was in acceptance with other reports (1,6,12,17) that the presence of EBNA was developed about four months after infection, and remain for life while EBEA appear during primary infection and considered an indicator of active infection and could be useful diagnostically.

We conclude that this cancer was present in Iraqi patients and EBV had involved in the development of carcinoma of the nasopharynx because those patients had a high levels of antibodies to EBV both EBVNA and EBVEA while control group did not had this antibodies. Other reports showed that antibodies were developed against viral capsid antigens of IgM type that appear early in the course of infection and of IgG type that was helpful in the diagnosis of infection (1,6,12). The presence of these antibodies in the serum of Iraqi patients suggested that EBV had involved in the development of this cancer in Iraqi patients. Other reports in other countries showed that EBV infection occur early in life, with immunity to EBV acquired primarily after four years (6,15). In addition to EBV specific antibodies, non-specific hetrophile antibodies were found that react with any components of EBV and disappear within six months after recovery (6). Studying cellular immunity in this revise, we found that CD8+ cytotoxic cells was significantly increased 27.56 +_ 2.02 and T lymphocytes (percentage of rosette-forming cells in the peripheral blood) and B lymphocytes percentages were decreased significantly (37.1+_ 1.76 and 3.33 +_ 0.25 respectively while CD4+ cells and CD4+/CD8+ ratio did not affected.

This may be due to EBV produced IL-10 which had IL-10 like activity and like IL-10 tends to suppress TH1 activity by cross-regulation and reduce the cell mediated response to EBV thus conferring a survival of the virus.

This reflect the role of TH1 / TH2 balance in determining the outcome of disease (17). This virus may also due to lymphopenia and suppression of cellular immunity cause it and certain degree of humeral responses detected by hyper gammaglobulinaemia in sera of some patients (5). All above disturbances in the results had no prognostic value in predicting the treatment response to chemotherapy (5).

In our study the increase in the CD8+ cells, this may be due to cytotoxic T cells recognize virally determined epitopes on infected cells make up the major effecter arm and control the infection.

This results was in agreement with other studies, which detected that there was a high number of peripheral activated CD8+ cells with low cytotoxicity (8), this was due to tumor cells were unable to process EBV antigens and presented to cytotoxic T cells presumably because of defect in antigen processing genes such as TAP1 and TAP2 genes (4). Other studies showed that an important regulatory mechanism for the maintenance of EBV latency in B-lymphocytes was T cells competition for growth factors produced and utilized by EBV immortalized B cells (14).

EBV encoded genes that ensure its persistence in human B-lymphocytes and encourage B-cell proliferation and evade immune recognition (9). Evasion from cytotoxic T lymphocytes surveillance might be an important step in the pathogenesis of Epstein-Barr virus by down regulation of all transformation associated viral antigens except EBNA-1and certain HLA class I alleles. EBNA-4 was the predominant target of HLA restricted cytotoxic T cells and EBNA-6 was the lesser target for these cells (10).

The decrease in the percentages of T and B cells were in agreement with Ware *etal* (11) who showed decrease in the number and increase in the expression of surface lymphotoxin and tumor necrosis factor on activated T, B (CD20+) and natural killer cells (CD56+) in peripheral blood. The long term T cell immunity to Epstein-Barr virus was considered to play an important role in suppressing proliferation of EBV infected B cells and out growth of EBV associated tumors (13).

So this reduction in the percentages of both T and B cells might be due to virus induced lymphopenia.

RECOMMENDATIONS:

Other antibodies to EBV antigens like IgA antibodies to EBV capsid antigens appear to be a useful screening test for early detection of nasopharangeal carcinoma using different new methods. Other cellular and immunological testing must be done to study the immune response in those patients. We recommend doing a control group with some patients with other forms of carcinoma of the head and neck.

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