

Serofrequency of Epstein Bar Virus among Non Hodgkin's Lymphoma in Sudanese patients attending Radiation and isotope Center

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Abstract:

Background: Non Hodgkin lymphoma (NHL) patients are highly positive for EBV, because it is an oncogenic virus which depend on expressing virus cancer gene and immortalize infected lymphocytes. EBV cause aggressive tumor by rapid growth and necrosis, there are few published data in Sudan, therefore our study aimed to figure out the frequency of EBV among NHL in Sudanese patients.

Method: This was descriptive cross sectional study included detection of EBV in 70 patients with NHL patients and 20 control individuals aged (11 – 80) years old with mean age (24) years old, conducted in Radiation and Isotope Center in Khartoum State Sudan. During period from August 2014 To March 2015, EBV serum marker (IgG) was detected using Enzyme Linked Immunosorbant Assay (ELISA).Generated data was analyzed by using SPSS program.

Result: Out of 70 patients 51 (72.9%) were positive for EBV IgG and 19(27.1) samples were negative, for positive samples 27 (52.9%) were males and 24 (47.1%) were females, while among control group 1 (5%) was positive and 19 (95%) were negative This study shown statistically non significant relationship between gender and

seropositivity of EBV (P.value =0.05) however there is significant difference between patients samples and control group (P.value = 0.00).

Conclusion: *The seropositivity of EBV among NHL patients were high. For further association study we recommended by using Polymerase Chain Reaction ,and further confirmation and monitoring with large scale samples are recommended.*

Key words: Epstein Bar Virus, Non Hodgkin lymphoma, ELISA, Radiation and Isotope Center -Khartoum.

Introduction:

Epstein Bar Virus (EBV) is DNA genome a ubiquitous contains about 172 kbp has a G+C content of 59% encodes about 100 genes, it's belong to the lymphocryptoviruse genus of gamma herpesvirinae sub family, which are well-known as tumor virus that express virus cancer genes and immortalize infected-lymphocytes ⁽¹⁾. There are two types (EBV-1 and EBV-2) based on differences in the latency nuclear antigen genes ⁽²⁾. The virus found throughout all human populations, with a prevalence of over 90% in adults ⁽³⁾. The EBV share a tropism for B lymphocytes, and have a propensity to oncogenicity, ⁽⁴⁾ well reported association with Non Hodgkin lymphoma ⁽⁵⁾, Most EBV-associated NHL are aggressive tumors characterized by rapid growth and necrosis ⁽⁶⁾.

Non Hodgkin lymphoma (NHL) is solid heterogeneous tumor affect B and T cell, arise primarily in lymph node ⁽⁷⁾. Worldwide, reports on the incidence of lymphomas are variable, with 60% of all childhood lymphomas being classified as NHL, representing 8% of all child hood malignancies, In Equatorial African countries, the most common type of lymphoma is NHL The EBV share a tropism for B lymphocytes, and have a propensity to oncogenicity, well reported association with Non Hodgkin lymphoma^(4,5).

The aim of this study is to figure out the frequency of EBV among Sudanese patient with Non Hodgkin lymphoma (NHL).

Materials and Methods:

This study was a descriptive cross-sectional study conducted in Radiation and Isotope Center in the period from August 2014 to March 2015.

A total number of 90 participants (70 patients with known NHL and 20 were control group) were included in this study. They were from both sexes, and their ages ranged from 11 to 80 years old. Patients with other Hodgkin lymphoma were excluded from this study. The data was collected using structured interview questionnaire.

Experimental work:

Specimen collection:

Two milliliter of venous blood was collected in plain container and centrifuged at 4000 revolution per minute for 5 minutes, to collect serum which used for detection of EBV antibodies (IgG) by using ELISA (EUROIMMUN , Germany) technique.

This study was approved by Al Neelain ethical committee and informed consent was taken from each patient before sample collection.

Procedure:

For semi quantitative analysis calibrator 2 was incubated along with the positive and negative controls and patient samples. Sample incubation: 100 µl of the calibrators positive and negative controls or diluted patient samples were transferred into the individual micro plate wells according to the pipetting protocol. Incubated for 30 minutes at room temperature washing: Automatic wash reagent wells 3 times with 450 µl working strength wash buffer. Wash buffer was left in each

well for 30 to 60 seconds per washing cycle, and then empty the wells. After washing, thoroughly were disposed of all liquid from the micro plate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffers.

Conjugate incubation:

100µl of enzyme conjugate was pipetted (peroxidase- labeled anti – human IgG) into each of the micro plate wells, incubated for 30 minutes at room temperature. Washing: Empty the wells, were wash as described above Substrate incubation: 100µl of chromogen/substrate solution was pipetted into each of the micro plate wells. Incubated for 15 minutes at room temperature protected from direct sun light). Stopping the reaction: 100µl of stop solution was pipetted into each of the micro plate wells in the same order and at the same speed as the chromogen/substrate was introduced. Measurement: Photometric measurement of the color intensity was made at wavelength of 450 nm and reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution.

The data was analyzed by using statistical package for social sciences (SPSS) version 16 for windows

Interpretation of results:

Results can be evaluated semi quantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2.

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio < 0.8:	Negative
Ratio ≥ 0.8 to <1.1:	borderline
Ratio ≥ 1.1:	positive

Results:

A total of 70 known diagnosed NHL patients were included in this study, 42 (60%) of patient were males and 28 (40%) were females, their age range from 11 years to 80 years (mean = 24 ±11 SD). and 20 normal healthy individual were also included as control 10 males and 10 females their ages range from 12 years to 55 years (mean 30 ±10.3 SD). The frequency of positive result of anti-EBV (IgG) among patient was 51 (72.9%) and 19 (27.1%) was negative(figure1) . Whereas the frequency among control group was 1 (5%) positive and 19 (95%) negative (figure 2). The posititivity of anti EBV (IgG) was higher in patients than control group which was significantly difference by chi square test. (Table 1), there was no significant differences of anti EBV (IgG) among gender of patients (*P. value 0.05*).

Table (1): Association between (NHL) patients and control group with EBV sero frequency.

IgG Results of EBV	Study group		Total	P. values
	Patient with NHL	Control		
Positive	51 (72.9%)	1 (5%)	52	0.000
Negative	19 (27.1%)	19 (95%)	38	
Total	70	20	90	

Figure: (1): Serofrequency of EBV among study population (n: 90) according to their gender of patients.

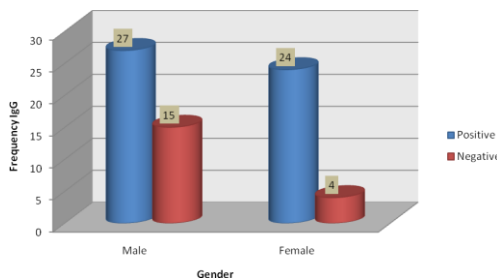
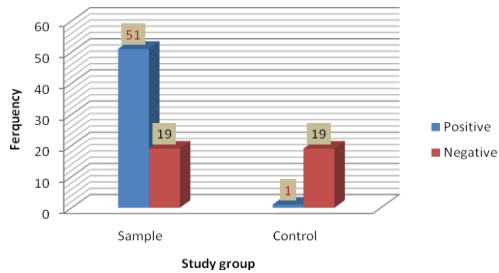


Figure (2): The frequency of EBV among patients (n: 70) and control (n: 20).



Discussion:

NHL is a worldwide distributed especially in Africa with many studies on going to figure out the association between EBV and non Hodgkin lymphoma ⁽⁵⁾. EBV is a member of the gamma sub family of herpes viruses is present in all human population, infecting greater than 95% of mankind within the first decade of life ⁽¹⁾. Infection in Africa and other developing areas was characterized by primary exposure in early child hood, perhaps due to certain cultural practice, than in the developed countries ⁽⁵⁾.

The current study was conducted in NHL patients. There were 90 participants in this study 70 Participants were Known NHL and 20 controls. In this Study there was high frequency of anti EBV (IgG) among the patients with NHL which represents about (72.9%).

When compared with other finding our study was in agree with the results of Epstein –Barr virus association with malignant lymphoma in Zaria, in Nigeria ⁽⁸⁾. Their results of the study were 55 cases of malignant lymphoma examined, 28 were males (50.9%) positive.

Worldwide, NHL is more in males than females, comparable results was found in current study in which 60% were males and 40% were females which in agreement with study done by Alwan A.F, et al ⁽⁹⁾ 2014 in which males were 64.2% and females were 35.8%, our results were similar to that in Iraqi results of EBV in NHL patients (78.6%) comparable

with our Positive results (72.9%). Additionally , different studies from the middle east have also reported a little bit lower incidence of EBV in NHL than our study, Oman study with 65% NHL⁽¹⁰⁾. Bahrain study with 66.7 % NHL ⁽¹¹⁾ and United Emirates with 59% NHL ⁽¹²⁾. From other side Sudan consider one of the developing countries. Developing countries represents high association between EBV and NHL as high as 80% ^(13, 14).

Conclusion:

High serofrequency of EBV was observed among study compared with control further researches must be conducted using advanced techniques to validate this result.

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