

Molecular Detection of Epstein Barr Virus among Multiple Myeloma Sudanese Patients in the Khartoum State

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Accepted 25 Oct 2016, Available online 30 Oct 2016, Vol.4 (Sept/Oct 2016 issue)

Abstract

Multiple myeloma is one of hematological malignancies, specifically lymphoproliferative disorders that characterized by normal plasma cell transformed to neoplastic plasma cell, The pad consequences follow the malignant transformation by proliferation of the neoplastic plasma cell, Previous studies describe the EBV interfering or modulating with cellular DNA repairing mechanisms and could lead to genetic changing and manipulation in the infected cells, The goal of this study was to establishing molecular based detection of the EBV in the Sudanese multiple myeloma patients by using polymerase chain reaction as one of nucleic acid amplification technology in the Khartoum-Sudan. This a descriptive, retrospective hospital based study conducted in molecular biology research center lab at The National Ribat university, and biomedical research lab, faculty of medical lab sciences, University of Khartoum, during the period between January 2014 to March 2016, thereby 89 clinically as much as histopathologically diagnosed cases regardless to genders were included. It is worthy mentioned that the archival specimens include both dried bone marrow aspirate and trephine blocks. DAN was extracted then amplified using PCR detection of BLLF1 gene. The present study included 37 of multiple myeloma cases designated as group I, whereas group II included 22 lymphoma cases and group III included 30 acute myelocytic leukemia patients, the diagnosis of these patients were confirmed based upon WHO criteria of each of multiple myeloma, lymphoma and acute myelocytic leukemia respectively. Conventional PCR was used for detecting EBV genome via targeting BLLF1 gene, the 37 multiple myeloma cases along with 30 cases of acute myelocytic leukemia were found to be negative $P = 0.10$, $P = 0.21$ correspondingly, whereas 22 cases of lymphoma were found to be positive for EBV genome, BLLF1 gene $P = 0.001$. Our study results may suggest that there is no possible association between EBV and multiple myeloma.

Keywords: Multiple Myeloma, Epstein Barr Virus, Polymerase Chain Reaction, Blf1 Gene, Sudanese

1. Introduction

The multiple myeloma is one of hematological malignancies, specifically, lymphoproliferative disorders that are characterized by deviation of normal plasma cell into neoplastic plasma cell, surprisingly, this transformation with unknown etiology. [1], [2], [3], [4] However, some may impute it to environmental factors, genetic factors or infectious agent factors. Basically, the plasma cells develop in the bone marrow as an immune specialized cell from B lymphocyte to play a paramount

role in producing antibodies to fight the specific antigen. [5] Nonetheless, ominous consequences follow the malignant transformation by exponential proliferation of the neoplastic plasma cell including; infiltration into the bone or other organs and excessive production of monoclonal immunoglobulin that result in presentation of signs or symptoms for patients with multiple myeloma. [6]

The global epidemiology of multiple myeloma is well known. Accumulated evidence has shown increasing incidence of the disease, it was estimated to be 4 to 4.5

per 100,000 populations every year. Furthermore, 63,000 patients are reported to die each year (33,000 males and 30,000 females), but in Sudan no published data are documented with increase of patients outcomes.[7]

Primarily, Epstein Barr virus [EBV] is a double stranded DNA virus according to Baltimore's classification. It belongs to Herpesviridae, also known as human herpesvirus [HHV4], interestingly, the EBV has a two target cells in the human, namely, B lymphocytes (entering through CD21) and epithelial cells (entering through cellular β 1 integrins and $\alpha\beta$ 6/ $\alpha\beta$ 8 integrins) with latency in the B lymphocytes by role of host immune system. BLLF1 gene is highly conserved and has more than 30 copies in the EBV genome, the EBV firstly virus described as carcinogenic infectious agent as the etiologically closely associated with the endemic Burkitt's lymphoma, Hodgkin disease, and nasopharyngeal carcinoma [8], [9], [10], [11]. Growing bodies of studies have described the EBV interfering or modulating with cellular DNA repairing mechanisms and could lead to genetic changes and manipulation in the infected cells. [9], [10] Furthermore, exhaustive studies have focused on the association of the EBV Infection with B-Cell lymphoproliferative diseases via investigating the seroprevalence of EBV among the patients with lymphoma and multiple myeloma as well.[12]

The current study was aimed at detecting EBV among multiple myeloma Sudanese patients using polymerase chain reaction.

2. Materials and Methods

A descriptive, retrospective hospital based study was conducted in molecular biology research center lab at National Ribat University, and biomedical research lab, faculty of medical lab. sciences, University of Khartoum, during the period of January 2014 to March 2016. In the context 89 clinically as much as histopathologically diagnosed cases, irrespective to genders as well as ages were included study. Not to mention that the archival specimens include both smear of dried bone marrow aspirate and trephine blocks of same patients, collected via random sample selection. Samples were collected from Asia hospital, Fedail private hospital and radiotherapy isotopes centre Khartoum (RICK). The studied populations were categorized into three groups by pathological conditions. Group I designated as cases includes 37 multiple myeloma patients. Group II include 22 samples of lymphoma patients and group III include 30 specimens of acute myelocytic leukemia patients, correspondingly.

Specimens were processed and total DNA extraction then afterward purification were performed via using QIAGEN kit (DNeasy 96 Blood and Tissue Kit, Germany) as follows dried smear of bone marrow aspirate was liquefied by making elute through using phosphate buffer saline of the smear then treated, and DNA extraction was proceeded. And by dewaxing paraffin from formalin

fixed trephine and clot embedded sections (5-10mm sections) in 1.5 ml eppendorf tube and then rehydrated by highly standard process then the DNA extraction was proceed according to manufacture instruction of DNeasy 96 Blood and Tissue Kit. Following extraction and purification the DNA was qualitatively measured by 1x Agarose gel to determine the integrity of the DNA; the highly sheared band was excluded and quantitatively by using Nano-Drop spectrophotometer, then stored in - 20° C till performing PCR. Sample with < 10 ng/ μ l from both specimens were excluded.

The polymerase chain reaction for detecting of EBV genome based on BLLF1 (glycoprotein 340) target gene was performed by using CinnaGen qualitative Epstein – Barr virus PCR detection kit Iran, and the procedure was followed according to the manufacture of instruction by using TC- 412 Techne (Bibby Scientific - UK) thermo cycler and result visualized by using gel electrophoresis and gel documentation system onto 2% Agarose gel to visualize 256 bp as positive result of EBV.

The ethical approval was obtained from the ethical committee of private and public hospitals.

Results data were statistically analyzed by using statistical package for social science (SPSS) computer program version 21, and qualitative statistic data were measured by Chi-square, significance by P value < 0.05 and insignificance by P value > 0.05.

3. Results

The studied populations include 89 cases. their ages ranged between 40 to 75 years with mean age of 57.5. Qualitative PCR procedure was designed to detect EBV genome via targeting BLLF1 gene, remarkably, the 37 cases of multiple myeloma beside 30 cases of acute myelocytic leukemia were found to be negative P = 0.10, P = 0.21 respectively. Whereas 22 cases of lymphoma were found to be positive for EBV genome, BLLF1 gene P = 0.001, these results are presented in table No. 1. The amplicon products of the EBV were visualized in the 2% Agarose gel stained with DNA safe stain show in figure 3.1.

Table 1: Shows EBV DNA positivity in the bone marrow aspirate and trephine with clot sections for the same patient

Study population & Gender	EBV DNA positivity	EBV DNA Negativity	P value
Group I (Multiple myeloma)	0	37	0.1
Group II (Lymphoma)	22	0	0.001
Group III (Acute myelocytic leukemia)	0	30	0.21
Total	22	67	89

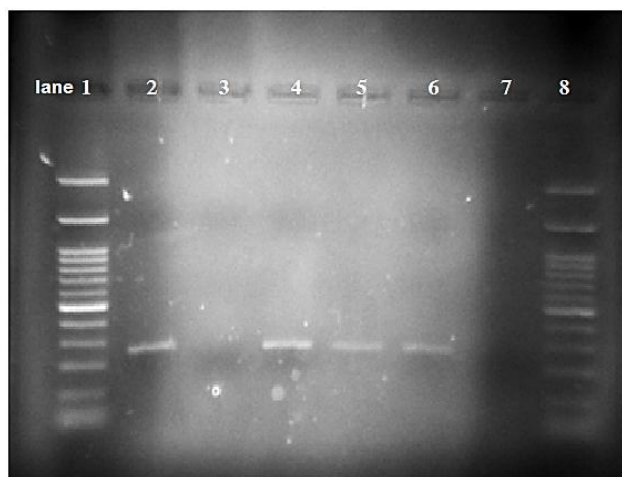


Figure 1: 2% Agrose gel showing the of EBV (BLLF1 gene)

PCR result, lane 1&8 displaying DNA molecular marker(100bp), lane 2 is showing positive band of EBV in the target region of BLLF1 gene in size 256 bp as positive control, lane 7 shows the negative control (ddH₂O with master mix), lanes 4,5 and 6 show positive results compared to positive control and lane 3 shows negative PCR for EBV.

4. Discussion

Multiple myeloma is one of special B lymphocytic malignancy disorders. Not surprisingly, transformation of the normal plasma cell to myeloma plasma cell with unknown etiology triggers multiple clinical consequences. Oddly strange, multiple myeloma as non-communicable disease. [1], [2], [3], [4]

The data presented in the current study showed insignificant association between the multiple myeloma and EBV infection $P = 0.10$ via examining the BLLF1 gene that highly conserved and have more than 30 copies in the EBV genome, nonetheless, the other hematological malignancy controls, B lymphocytic lineage (lymphoma) exhibited significant association between EBV and lymphoma $P = 0.001$, whereas myelocytic lineage (acute myelocytic leukemia) showed insignificant association between EBV and acute myelocytic leukemia $P = 0.21$, it is worthy mentioned that all of them were done from two patient materials. Our findings were in accordance with study by Vega *et al.* [13], who has reported a significant difference in EBV DNA between plasmablastic lymphoma and plasma cell myeloma. Remarkably, EBV was positive in all patients with plasmablastic lymphoma but not in any cases of plasma cell myelomas. Furthermore study by Minowada and his colleague's have no found association between EBV and multiple myeloma [14]. Additionally mounting evidence of study has reported no significant difference between EBV seroprevalence in patients with multiple myeloma.

In contrary study by Sadeghian *et al* [15] has detected EBV DNA in the formalin-fixed paraffin embedded (FFPE) bone marrow biopsies of total 60 FFPE of multiple

myeloma patient, importantly, their results data show significant association between the EBV infection and multiple myeloma $P = 0.01$, other studies were show 100% association between EBV and Non-hodgkin's lymphoma but the theoretically there is no association of EBV with acute myelocytic leukemia. Moreover, our study results were contradicted with Csireet *al* [16]. Their estimated the presence of EBV in patients with multiple myeloma using both serologic based (phenotypic) and molecular based (genotypic) techniques the virus genome was detected in 36 (52%) of 69 multiple myeloma patients $P < 0.05$.

Conclusions and Recommendations

It is well evident that EBV plays no significant role in the initiation, and development of multiple myeloma as much as acute myelocytic leukemia. In contrary EBV could be indicated as possible potential etiological agent in causing lymphoma, we recommend to establishing EBV RNA based study.

Acknowledgment

We would like to express of thanks to members of the faculty of medical laboratory sciences, University of Khartoum, Ribat research center at The National Ribat University for their tireless help and support.

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