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RESEARCH ARTICLE

PCR Detection for Epstein-Bar Virus Associated with Leukemia Patients in Babylon Province of Iraq

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Abstract

The first virus to be implicated in the causation of a human cancer was the Epstein–Barr virus (EBV), discovered in 1964 by Epstein, and Barr in a lymphoid cell line established from a biopsy of an African Burkitt lymphoma. The present study establish for detection Epstein-Bar virus in thirty serum samples collected from Marjan hospital in Babylon Province of Iraq. Detection of this virus conducted by polymerase chain reaction for blood diagnosed firstly as having Epstein-Bar virus with leukemia by ELISA technique. Twenty three from thirty samples are positive for EBV association with leukemia patients according to PCR produced 161 bp band referred to EBV gene noticed at gel electrophoresis technique.

Keywords: *Epstein–Barr virus, Leukemia, PCR.*

Introduction

Epstein-Barr virus (EBV is one of the herpesviridae family of the most common viruses in humans, composed of double helix DNA which contains about 172000 base pairs and 85 genes, the DNA surrounded by nucleocapsid protein with approximately 122–180 nm in diameter [1].

This nucleocapsid is surrounded by a tegument made of protein, which in turn is surrounded by an envelope containing both lipids and surface projections of glycoproteins which are essential to infection of the host cell, that best known as the cause of infectious mononucleosis (glandular fever) [2]. It is also associated with particular forms of cancer, such as Hodgkin's lymphoma, Burkitt's lymphoma, gastric cancer, nasopharyngeal carcinoma, and conditions associated with human immunodeficiency virus (HIV), such as hairy leukoplakia and central nervous system lymphomas [1.3].

The evidence to infection with EBV is associated with a higher risk of certain autoimmune diseases, especially dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis [4]. Infection with EBV occurs by the oral transfer of saliva and genital secretions [5].

As previously stated there are few symptoms or asymmtomatic when a person contracts EBV at the adolescence stage of life but when EBV is contracted as an adult it may cause fatigue, fever, inflamed throat, swollen lymph nodes in the neck, enlarged spleen, swollen liver, or rash [6]. Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-old children and about 90 percent of adults have evidence of previous infection; Infants become susceptible to EBV as soon as maternal antibody protection disappears [7].

Aims of the Study

Because there is no related study in Iraq, therefore this study aimed to detect the EBV gene with PCR technique in leukemia patients in Babylon province of Iraq.

Material and Methods

Samples Collection

This study includes 30 patients with Chronic Myeloid Leukemia (CML) admitted to Marjan Hospital in Babylon Province of Iraq. Patients were diagnosed by specialist physicians and selected in the current study.

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Blood Sampling

About two milliliters of venous blood were collected from each patient in the study

Isolation of Genomic DNA

Genomic DNA was used for molecular study by sequestered from the fresh blood, which collected in tubes of anticoagulant EDTA and for frozen blood samples we recommended using protease K were applied using for DNA purification; Promega Wizard genomic kits. The isolation of DNA depended on the 5 stage procedure utilizing salting out techniques [8]:

- Lysis of the RBCs in the Cell Lysis Solution.
- Lysis of the WBCs and their nuclei in the Nuclei Lysis Solution.
- The cellular proteins were then removed by a salt out precipitation step using the Protein Precipitation Solution.
- The genomic DNA was concentrated and desalted by Isopropanol precipitation.
- The genomic DNA was rehydrated using the DNA Rehydration Solution.

Isolation Kit Components

Components Amount

Cell Lysis Solution 500 ml Protein Precipitation Solution 125 ml

Nuclei Lysis Solution 250 ml DNA Rehydration Solution 100 ml

The Protocol for DNA Separation

Procedure which provided with Promega kits recommend for DNA separation as reveled in bellow:

- Cell Lysis Solution (900 µl) was added to a clean 1.5 ml small scale rotator tube.
- Gently shook the container of blood until it was altogether blended; then 300 µl of blood was transfused to the tube which contained the cell lysis arrangement. The tube was modified 5-6 times to be blended.
- The blend was hatched for 10 minutes at room temperature and modified 2-3 times amid brooding to lyse the RBCs. In addition, the tube centrifuged at 13000-14000 rpm/20 seconds at room temperature.
- The supernatant was expelled and disposed of however much as could be expected without exasperating the obvious white pellet.
- The tube was vortexed enthusiastically until the WBCs were re-suspended (10-15 seconds).
- Nuclei Lysis Solution (300 µl) was added to the re-suspended cells; the arrangement was pipetted 5-6 times to lyse the WBCs. The arrangement ought to have turned out to be exceptionally gooey. If clusters of cells were unmistakable in the wake of blending, the arrangement ought to have been brooded at 37oC until the bunches interruption; if clusters are still obvious following 60 minutes, extra Nuclei Lysis Solution (100 µl) ought to be included; in addition, the hatching ought to be rehashed.
- Protein Precipitation Solution (100 µl) was added to the atomic lysate and vortex enthusiastically for 10-20 seconds. Little protein bunches may be obvious.
- The tube was centrifuged at 13000-14000 rpm for 3 minutes at room temperature until dull cocoa protein pellet was noticeable.
- The supernatant was exchanged to a clean 1.5 ml smaller scale rotator tube which contained 300 µl of room temperature Isopropanol; in addition, the arrangement was tenderly blended by reversal while waiting for the white string approximating features of DNA frame noticeable mass.
- Centrifugation was directed at 13000-14000 rpm for 1minute at room temperature; the DNA was obvious as a little white pellet.
- The supernatant was emptied, and one specimen volume of room temperature 70% Ethanol was added to the DNA. The tube was delicately modified little periods to wash-down the pellet of nucleic acid and the adjacent of the slighter scale rotator tube. Centrifugation as is specified in step 10.
- The Ethanol was deliberately suctioned utilizing a micropipette .The DNA pellet was free by then; along these lines, care must be taken to abstain from suctioning the pellet into the pipette. The tube was

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upset on clean retentive paper and air-dried the pellet for around 10-15 minutes.

- The DNA Rehydration Solution (100 µl) was added to the tube and the DNA was rehydrated by bringing forth it's at 65°C for an hour. Irregularly, the game plan was mixed by tapping the tube carefully. Then again, the DNA was rehydrated by hatching the arrangement overnight at room temperature or at 4 °C.
- DNA was put away in a cooler at - 20 °C.

The Estimation of DNA Concentration and Purity:

The DNA concentration of samples was estimated by using the Nano drop by putting 2.5µl of the extracted DNA in the machine to detect concentration in ng/µL and the purity detected by noticing the ratio of optical density (OD) 260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ration for purifying DNA was between 1.7-1.9 [9].

Electrophoresis of Agarose Gel

Agarose gel electrophoresis was embraced to affirm the nearness and uprightness of the separated DNA after genomic DNA extraction in 1.5% agarose with 100 V for 10 min [8].

Gel Electrophoresis Reagents:

- Powder of Agarose
- TBE Buffer with 1X concentration
- Loading dye
- Ethidium Bromide
- DNA Ladder Marker

Protocol of Gel Electrophoresis

Tris Borate EDTA Buffer preparation (1X TBE)

This solution was prepared by adding 900 ml distilled water to 100 ml 10X TBE (Promega/ Germany), forming 1 liter of(1x) TBE buffer [8].

Preparation of Agarose Gel

- The amount of 1 X TBE (100 ml) was taken in a beaker.
- Agarose powder (1.5 gm) was added to the buffer.
- The solution was heated to boiling using a microwave oven for 2 min.
- Ethidium Bromide (1 µl) of (10mg/ml) was added to the agarose solution.
- The agarose was stirred in order to be mix and avoid making bubbles.
- The solution was left to cool down at 50-60 °C.

DNA Loading & Electrophoresis

3µl of DNA was mixed with 2µl loading dye. The samples loaded carefully into the individual wells of the gel, and then electrical power was turned on at 70 volt for 1hour, afterwards the DNA moved from cathode (-) to anode (+) poles. The Ethidium Bromide stained bands in the gel were visualized using UV. Transilluminator at 350 nm and photographed.

PCR Technique

Tables 1 show the steps of PCR program technique that used to detect the presence of EBV virus. EBV-LMP1-DNA sequences were amplified by Polymerase PCR by lyophilized forward: 5′CGGAAGAGGTTGAAAACAAA3′ and Reverse: 5′GT GG GG GT CG TC AT CA TCTC3′ primers supplied by Bioneer (Korea) Organization as a result of various picomols fixations [10]. Lyophilized preliminary was disintegrated in a free DNase/RNase water to give a final concentration of100 pmol/µl and kept as a stock in -20 °C, to prepare 10µM concentration as work primer re suspended 10 pmol/µl in 90 µl of free DNase/RNase to reach a final concentration 10µM.

Detection of EBV Virus

Table1: PCR detection steps to EBV Virus

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	94	5	1
Denaturation	94	1	35
Annealing Zones	55	1	35
Extension	72	2	35
Final extension	72	7	1
Storage	10	∞	

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Results

Twenty three from thirty samples are positive for EBV association with leukemia patients by PCR technique.

The results of current study revealed gene (161bp) of Epstein-Bar virus in leukemia patients (Figure 1).

Figure 1: Detection of Epstein-Bar virus in leukemia patients by polymerase chain reaction technique

Discussion

EBV turned into the prime case of a human tumor infection that is etiologically connected to an assorted scope of malignancies. EBV has been connected with an assortment of lymphoid and epithelial malignancies [11]. Leukemia, as various tumors, results from physical changes in the DNA. Certain progressions produce leukemia by inciting oncogenes or deactivating tumor silencer qualities, and thusly disquieting the bearing of cell end, partition or division. These progressions may happen all of a sudden or as an outcome of prologue to radiation or malignancy bringing about substances, and are inclined to be affected by inherited segments [12]. Among adults, the known causes are typical and fake ionizing radiation, several contaminations, for instance, Human T-lymphotropic disease, Epstein-Bar contamination, Cytomegalovirus and a couple of chemicals, prominently benzene and alkylating chemotherapy masters for past malignancies [13]. The ingenuity of EBV and its relationship to tumorigenesis was set up [14, 15]. Three main pieces of evidence have been used to show, separately or in conjunction with each other, that EBV must have been present in tumor progenitor cells before their malignant conversion.

First, in some rare instances, EBV has actually been demonstrated in pre-malignant lesions related to a specific cancer showing that infection is an early event in the development of the minor [16]. Second, all malignant cells within a tumor contain EBV DNA. Third, the EBV DNA represents a homogeneous episomal population demonstrating that the maugnancy is likely a clonal development of a solitary EBV-infected ancestor cell [17].

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