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DETECTION OF EPSTEIN–BARR VIRUS AS A POSSIBLE VIRAL CAUSES OF INFECTION MONONUCLEOSIS BY USING POLYMERASE CHAIN REACTION IN SUDANESE PATIENT PRESENTING TO MAJOR HOSPITALS IN KHARTOUM STATE, SUDAN

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ABSTRACT

Background: Epstein–Barr virus is members of the human herpesviruses, is common virus that cause infectious mononucleosis (IM) characterized by fever, pharyngitis and lymphadenopathy, EBV infects at least 90% of the world's population and can persist in a latent form after primary infection. EBV shares with other herpesviruses the property of initial infection of young hosts, establishment of latency and "reactivation" later in life, with variable consequences. The present study conducted to determine the possible viral causes of infectious mononucleosis in Sudanese patients attending major hospital in Khartoum State, Sudan. **Materials and Methods:** Under aseptic condition, 4 mls of venous blood were collected in sterile EDTA container from 90 patients, 49 with infectious mononucleosis and 41 as a negative control. Then a thin blood film was performed and stained with rapid stain, after that the blood was centrifuged at 1500 RPM for 5 min to obtain plasma the plasma was then transferred to a sterile to eppendorf tube for detection of DNA genome by using PCR (Conventional PCR). **Results:** The rate of detection of EBV DNA was detected in 11(12.2%) of the cases. Females were more susceptible to be infected with EBV in our study 48 (12.50%) than males 42 (11.90%) The rate of detection EBV genome was higher (16.6%) in the age group more than 50 years. EBV genome detection was slightly higher in females compared to males (12.5% versus 11.9%) respectively. The percentage of EBV detection was higher among patients from Khartoum (12.8%). Using

polymerase chain reaction assay has facilitated the rapid detection of EBV DNA in individual specimens and also cost-effective and provides a wider differential diagnosis than do individual assays.

KEYWORDS: Epstein–Barr virus, EBV, herpesviruses, PCR, Infectious mononucleosis.

INTRODUCTION

Epstein Barr Virus also Human herpesvirus 4 (HHV-4) is a human pathogen, enveloped, double-stranded DNA virus of the family Herpesviridae. It was first described in 1964 by Michael Epstein and Yvonne M. Barr. They discovered EBV in B lymphocytes of African patients with Burkitt'slymphoma. Infectious mononucleosis was first described by Sprunt and Evans in the Bulletin of the Johns Hopkins Hospital in 1920. They described the clinical characteristics of Epstein-Barr virus (EBV) infectious mononucleosis. At the time, their article was entitled "Mononuclear leukocytosis in reaction to acute infection (infectious mononucleosis)," because the causative organism, EBV, had yet to be described.[1] There are several forms of Epstein–Barr virus infection. Infectious mononucleosis, nasopharyngeal carcinoma, and Burkitt's lymphoma can all be caused by the Epstein–Barr virus. Epstein–Barr can cause infectious mononucleosis, also known as 'glandular fever', 'Mono' and 'Pfeiffer's disease'. Infectious mononucleosis is caused when a person is first exposed to the virus during or after adolescence. Though once deemed "The Kissing Disease," recent research has shown that transmission of Mono not only occurs from exchanging saliva, but also from contact with the airborne virus. It is predominantly found in the developing world, and most children in the developing world are found to have already been infected by around 18 months of age. EBV antibody tests turn up almost universally positive. In the United States roughly half of five-year-olds have been infected.[2]

Infectious mononucleosis (IM; also known as EBV infectious mononucleosis, Pfeiffer's disease, Filatov's disease and sometimes colloquially as the kissing disease from its oral transmission or simply as mono in North America and as glandular fever in other English-speaking countries). It is an infectious, widespread viral disease caused by the Epstein–Barr virus (EBV), one type of herpes virus, to which more than 90% of adults have been exposed.[3] Epstein-Barr virus (EBV) is an important human pathogen with a worldwide distribution— one of the most common viral infection in humans (Masucci and Emberg, 1994). Most of the population in developed countries acquires immunity to EBV during childhood and adolescence (Schuster and Kreth, 1992).[4] EBV is transmitted oro-orally but

there have been conflicting findings about the most important reservoirs for infection. Different studies have found that same sex siblings (Lay and Mackat-Scollay, 1975).[5] Occasionally, the symptoms can recur at a later period.[6] Most people are exposed to the virus as children, when the disease produces no noticeable or only flu-like symptoms. In developing countries, people are exposed to the virus in early childhood more often than in developed countries. As a result, the disease in its observable form is more common in developed countries. It is most common among adolescents and young adults. The syndrome was described as an infectious process by Nil Filatov in 1887 and independently by Emil Pfeiffer in 1889.[7, 8] Acquisition of EBV in childhood is associated with Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, and possibly other malignancies (Masucci and Emberg, 1994).[9] It is also a candidate for an infectious cause of childhood leukemia. The epidemiology of EBV in children is therefore an important area for investigation (Kinlen, 1995).[10]

MATERIALS AND METHODS

Specimen collection and processing

Under aseptic condition, 4 mls of venous blood were collected in sterile EDTA container. Then a thin blood film was performed and stained with rapid stain, after that the blood was centrifuged at 1500 RPM for 5 min to obtain plasma the plasma was then transferred to a sterile to eppendorf tube. All plasma specimens were stored and preserved in a deep-freezer at -20^{o}C until processing.

DNA extraction

DNA was extracted from plasma by Cinna Pure DNA (Cinna Gen, Iran) kit that is specified in extracting of DNA from blood.

100µl of sample(plasma) was added to a sterile 1.5 ml polypropylene Tube, 400µl lysis buffer was added and vortexed at max speed for 20 seconds, 300µl precipitation solution was added and vortexed at max Speed for 5seconds, the solution was transferred to a spin column with collection tube by pipetting, the tube was centrifuged at 13.000 rpm for 1 min, collection tube discarded, column was span and centrifuged at 13.000rpm for Tube, the spin column was washed with 400µl of wash buffer II by centrifugation at 13.000 rpm for 1 min, the centrifugation step was repeated and Carefully the column to a new 1.5ml tube And add 400ul wash buffer I was transferred to the buffer in the center of the column, lid was closed

and incubated for 3-5 min at 65°C, Thereafter centrifuged at 13.000rpm for 1 min to elute the DNA

Primer design: EBV primers were designed by (MacroGen, Korea) that was as following:
Forward: 5′CTTAGAATGGTGGCCGGGCTGTAAAAT3′.
Reverse: 5′ ATCCAGTACGTCTTTGTGGAGCCCAAG3′(229pbs)

Polymerase Chain Reaction

The master-mix used in PCR was *Maxime* PCR PreMix Kit(*i*-Taq).Template DNA and primers were added into *Maxime*PCR PreMix tubes (*i*-Taq). 5-20pmol of primer/mleach (sense and anti-sense), distilled water was added to into the tubes to a total volume of 20ml.

PCR was started with initial denaturation at 94°C for 2 minutes, PCR cycles were done by denaturation at 94° C for 20 seconds, annealing at 60° C for 10 seconds and extension at 70°C for 25 seconds. Final cycle extension was in 72°C for 5 minutes. The PCR product was run in agarose gel electrophoresis and loading dye was added to the ladders. DNA was visualized using gel documentation system.

RESULTS

In the present study we aimed at analyzing the EBV DNA detection in plasma samples and the occurrence of EBV in infectious mononucleosis patients by means of a standard non quantitative PCR protocol (Conventional PCR) which is able to detect100 viral copies of a specific fragment of the expected size when using the primer set specific to the EBV (256bp) (Figure 1).

In this study a total of 90 subjects were included, 49 of them were atypical lymphocytes in their peripheral blood film and 41 as negative control (Table 1).

Out of these 42 (11.90%) males and 48 (12.50%) female were included, EBV genome detection was slightly higher in females compared to males (Figure 2).

EBV DNA was detected in 11 (12.2%) of cases (Figure 2). The percentage of the EBV genome was higher (16.6%) in the age group more than 50 years (Table 2).

EBV viruses detection was higher among patients from Khartoum (12.8%) (Tables 3). EBV was slighlty higher in patients with swollen lymph node 8 (22.8%) (Figure 3).

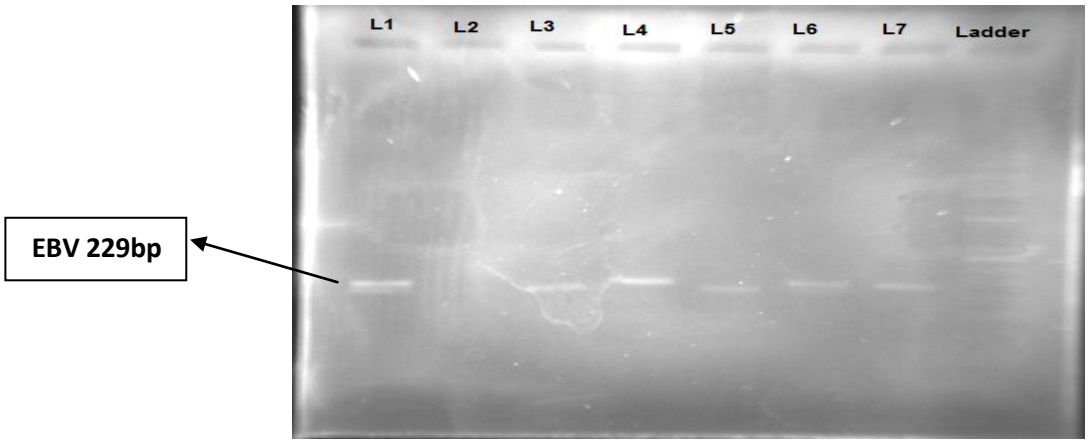

Figure (1): Show DNA ladder of molecular marker [100bp], Lane 1 show band of control positive for EBV [229bp] lane 2 show negative control for EBV, and lane 4, 6 & 7 show positive for EBV [229bp].

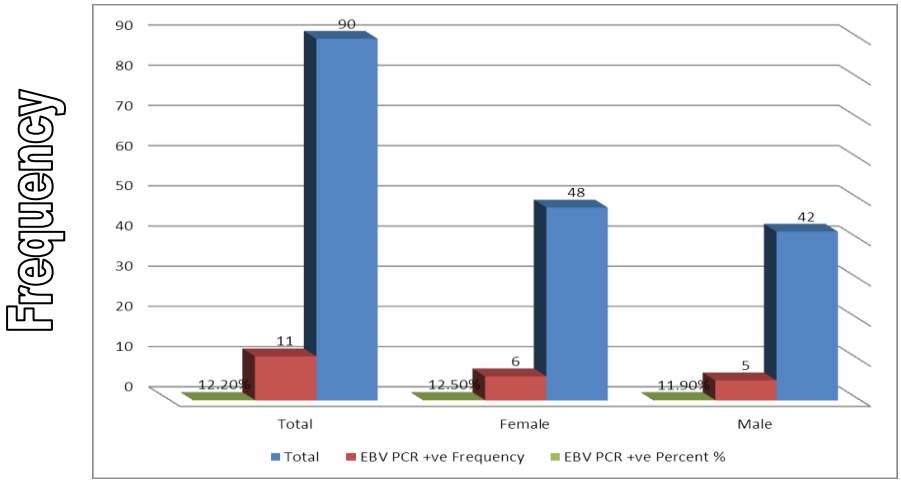

Figure (2): Distribution of Epstein-Barr virus according to gender.

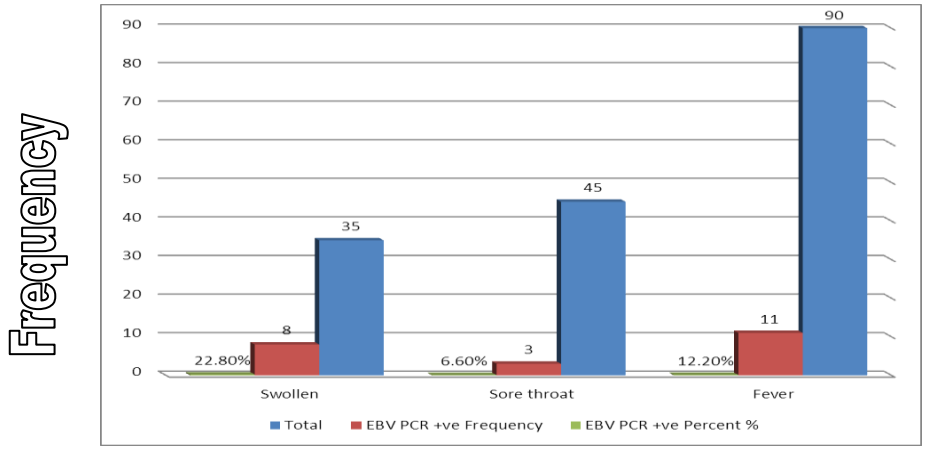

Figure (3): Distribution of Epstein-Barr virus according to symptoms.

Table (1): Distribution of Epstein-Barr virus according to atypical lymphocytosis.

Atypical lymphocyte in PBF	Total	EBV PCR +ve		P. value
		Frequency	Percent %	
Presence	49	8	16.3%	0.428
Absence	41	3	7.3%	
Total	90	11	12.2%	

Table (2): Distribution of Epstein-Barr virus according to age group.

Age group (Years)	Total	EBV PCR +ve	
		Frequency	Percent %
1-10	27	3	11.1%
11-20	12	1	8.3%
21-30	20	2	10%
31-40	13	2	15.3%
41-50	6	1	16.6%
Up to 50	12	2	16.6%
Total	90	11	12.2%

Table (3): Distribution of Epstein-Barr virus according to residence

Residence	Total	EBV PCR +ve	
		Frequency	Percent %
Khartoum	39	5	12.8%
Bahri	51	6	11.7%
Total	90	11	12.2%

DISCUSSION

In the present study Epstein Barr virus DNA in 11 (12.2%). Use of PCR has facilitated the detection of virus. There was no significant variation in the rate of infection with EBV between males and females in our study (p = 0.428). The rate of infection with CMV obtained in this study (10%) was in disagreement with that obtained by Druce*et al*. in 2002 (85%) and this may be due to the large sample size they analyzed during their study. Our finding found that the EBV DNA was found to be lower (12.2%) compared to that reported by Chan *et al.* in 2001 which were found to be higher (83%) (Chan *et al*., 2001). Our finding disagreed with study reported by Druce*et al*. (30-40 years) (Druce*et al.*, 2002). EBV genome detection was higher (90%) in the study reported by Gallagher *et al* in 1999, compared to that obtained in our study (12.2%) (Gallagher *et al.*, 1999). Regarding the detection of EBV genome there was no variation on the infection with EBV in females compared to males (12.5% vs. 11.9%) (p = 0.452).

CONCLUSION

In conclusion the rate of EBV genome detection was slightly higher in females compared to males (12.5% versus 11.9%) respectively. And low incidence of co-infection with EBV in patients suspected to have infectious mononucleosis, the use of PCR has facilitates the detection of virus. Therefore further in depth studies including large sample size and other areas are recommended.

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