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

Moustafa B. Ahamed[1]* and Khalid A. Abdelhalim[2]

[1,2]Department of Microbiology, Faculty of Medical Laboratory Sciences, National Ribat University.

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***Corresponding Author**
Dr. Moustafa B. Ahamed
Department of Microbiology, Faculty of Medical Laboratory Sciences, National Ribat University.
mustafaalshater@yahoo.com

ABSTRACT

Background: Cytomegalovirus is members of the human herpesviruses, the initial infection with CMV is usually asymptomatic, or causes a febrile illness, but can rarely manifest itself. Infectious mononucleosis is a clinical syndrome that is most commonly associated with (CMV) infection and other infectious agents, and may be associated with several acute complications. 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 Cytomegalovirus DNA was detected in 9 (10%) of the cases. Females were more susceptible to be infected with CMV in our study 48 (12.50%) than males 42 (11.90%). The rate of detection CMV genome was higher (16.6%) in the age group (11-20 years). The percentage of detection of CMV genome was slightly higher in females compared to males (8.3% versus 11.9%) respectively. CMV detection was higher among patients from Khartoum (12.8%). Using non quantitative

PCR assay has facilitated the rapid detection of viruses in individual specimens and also cost-effective and provides a wider differential diagnosis than do serological assays.

KEYWORDS: Cytomegalovirus, CMV, herpesviruses, PCR, Infectious mononucleosis.

INTRODUCTION

From the Greek cyto- "cell" and -megalo- "large" is a viral genus of the viral family known as Herpesviridae or herpes viruses. It is typically abbreviated as CMV. The species that infects humans is commonly known as human CMV (HCMV) or human herpesvirus-5 (HHV-5), and is the most studied of all cytomegaloviruses.[1] Within Herpesviridae, CMV belongs to the Betaherpesvirinae subfamily, which also includes the genera Muromegalovirus and Roseolovirus (HHV-6 and HHV-7) (Yamanishi*et al.*, 2007). It is related to other herpes viruses within the subfamilies of Alphaherpesvirinae that includes herpes simplex viruses (HSV-1 and -2) and Varicella-Zoster virus (VZV) and the Gammaherpesvirinae subfamily that includes Epstein–Barr virus.[1] All herpes viruses share a characteristic ability to remain latent within the body over long periods. Although they may be found throughout the body, CMV infections are frequently associated with the salivary glands in humans and other mammals.[2] Whereas most CMV infections of infants and children are asymptomatic or have non-specific symptoms, infections of adolescents and adults frequently result in infectious mononucleosis.[3,4] A lot of studies were conducted to detect CMV among different types of patients.[5,6,7] The cytomegalovirus (CMV) has become an extremely important pathogen, and review of its history is pertinent. CMV has now become one of the most common opportunistic pathogens encountered in patients immunocompromised from congenital or acquired causes such as AIDS or transplantation procedures.[8] Worldwide - Cytomegalovirus (CMV) is a universally distributed pathogen with approximately 40-100% of the world's population having CMV antibody present in blood as evidence of infection.[9,10,11] The risk of getting cytomegalovirus (CMV) through casual contact is very small. The virus is generally passed from infected people to others through direct contact with body fluids, such as urine, saliva, or breast milk. CMV is sexually transmitted. It can also be spread through transplanted organs and blood transfusions.[12]

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 (CinnaGen, Iran) kit that is specified in extracting 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^{o}C, Thereafter centrifuged at 13.000rpm for 1 min to elute the DNA.

Primer design

CMV primers were designed by (MacroGen, Korea) that was as following:

CMV: Forward: 5′GCGCGTACCGTTGAAAGAAAAGCATAA 3′

Reverse: 5′TGGGCACTCGGGTCTTCATCTCTTTAC 3′(131pbs).

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^{o}C for 2 minutes, PCR cycles were done by denaturation at 94^{o}C for 20 seconds, annealing at 60^{o} C for 10 seconds, and extension at 70 ^{o} C for 25 seconds. Final cycle extension was in 72^{o}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 sys.

RESULTS

In the present study we aimed at analyzing the CMV DNA detection in plasma samples and the occurrence of CMV 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 CMV (131bp) (Figure 1).

Ninety individual in this study were included, 49 of them were atypical lymphocytes in their peripheral blood film and 41 as negative (Table 1). Out of these 42 (46.7%) males and 48 (53.3%) females were included (Figures 2).

Cytomegalovirus DNA was detected in 9 (10%) (p=0.165) of the cases, (8.3%) for female and (11.3%) for male (P=0.021). The rate of detection CMV genome was higher (16.6%) in the age group (11-20 years) (Table 2).

8.50% of patients complained from swollen,(6.6%) of patients complained from sore throat, and (10%) of patients complained from fever were positive for CMV (P=0.428) (Figure 3).

The percentage of detection of CMV genome was slightly higher in females compared to males (8.3% versus 11.9%) respectively (Figure 2). CMV virus detection was higher among patients from Khartoum (12.8%) (Tables 3).

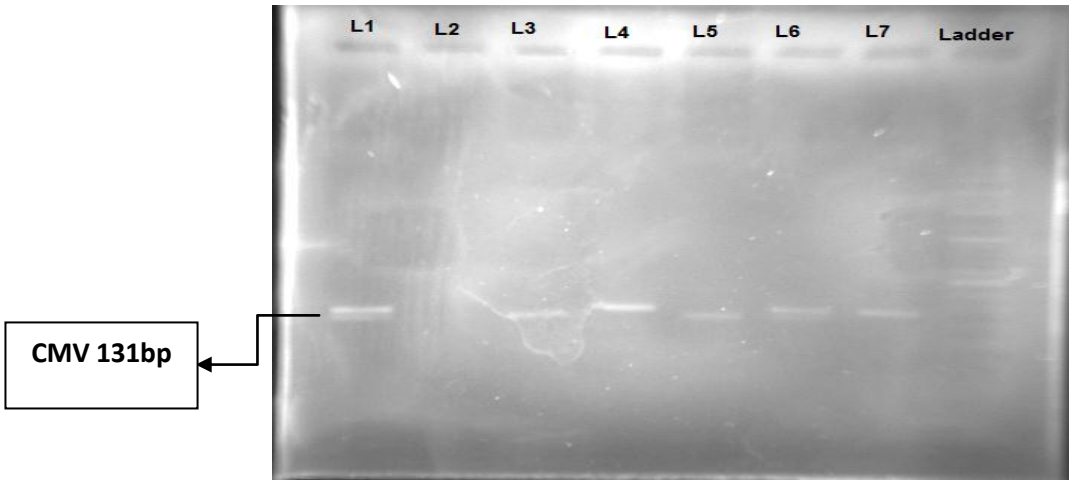

Figure (1): Show DNA ladder of molecular marker [100bp], Lane 1 show band of control positive CMV [131bp], lane 2 show negative control for CMV, lane 3, 5 & 6 show positive for CMV [131bp].

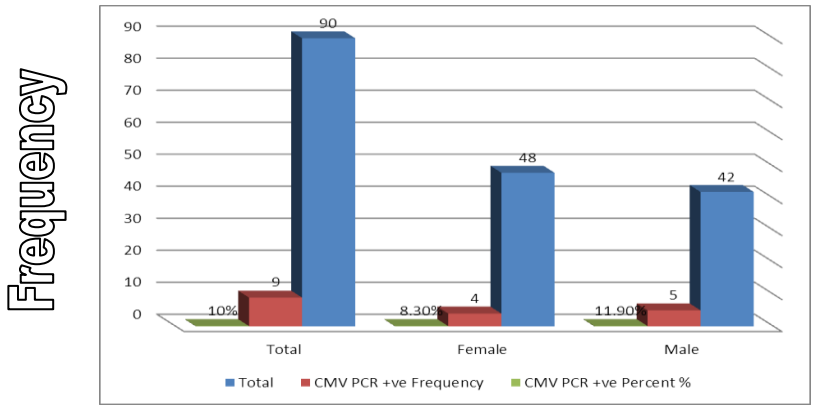

Figure (2): Distribution of Cytomegalovirus according to gender.

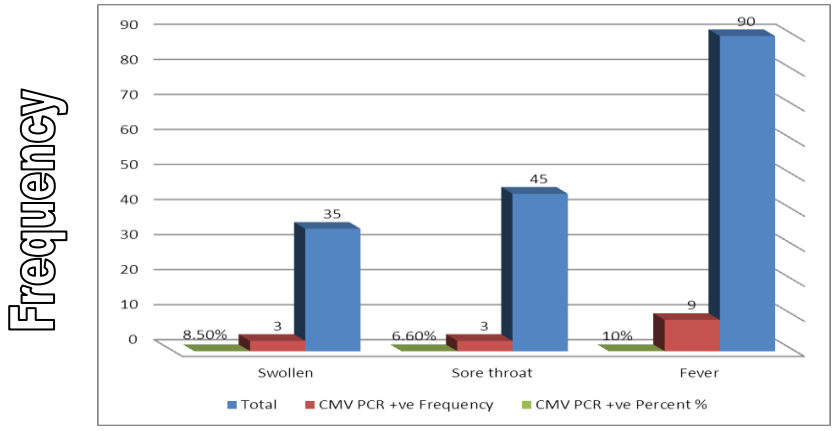

Figure (3): Distribution of Cytomegalovirus according to symptoms

Table (1): Distribution of Cytomegalovirus according to atypical lymphocytosis.

Atypical lymphocyte in PBF	Total	CMV PCR +ve		P. value
		Frequency	Percent %	
Presence	49	7	14.2%	0.428
Absence	41	2	4.8%	
Total	90	9	10%	

Table (2): Distribution of Cytomegalovirus according to age group.

Age group (Years)	Total	CMV PCR +ve	
		Frequency	Percent %
1-10	27	3	11.1%
11-20	12	2	16.6%
21-30	20	3	15%
31-40	13	1	7.6%
41-50	6	0	0%
50>	12	0	0%
Total	90	9	10%

Table (3): Distribution of Cytomegalovirus according to residence.

Residence	Total	CMV PCR +ve	
		Frequency	Percent %
Khartoum	39	5	12.8%
Bahri	51	4	7.8%
Total	90	9	10%

DISCUSSION

In the present study Cytomegalovirus DNA was detected in 9 (10%) of the cases. There was significant variation in the rate of infection with CMV in females compared to males (8.3% versus 11.9%) (p = 0.021). 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. The percentage of detection CMV genome was significantly higher (16.6%) in the age group (11-20 years) (p = 0.241). Our finding disagreed with study reported by Druce *et al*. (30-40 years) (Druce *et al.*, 2002). There was significant variation on detection of CMV genome on the patients from Khartoum (12.8%) compared to that detected in the patients from Bahri (7.8%) (p = 0.021).

CONCLUSION

CMV was detected in (14.2) % of patient with atypical lymphocyte compared to without atypical lymphocyte (4.8%). There was no significant difference between patient with or without atypical regarding the detection of CMV. 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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