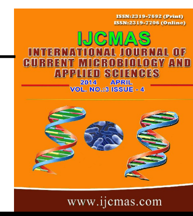

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Original Research Article

Molecular Detection of Human Cytomegalovirus genes in infertile and breast cancer women in Baghdad province

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Keywords

Viral genes *(UL55, UL97)*; Infertility Clinic; PCR technique

A B S T R A C T

This study was conducted to estimate the prevalence of two viral genes*(UL55, UL97)* of the virus among infertile and breast cancer women. Blood samples were collected from 128 out patients the age ranged (16-45 years, Mean±SD 30.5±8.8), serologically diagnosed as virus infected at the Infertility Clinic and Radiation Hospital in Baghdad during period from March 2012 to October 2012. These women included 98 infertile and 30 breast cancer women. Another fifty (50) blood samples were collected from apparently healthy women from same clinics and age range. Genomic and viral DNA was extracted from blood samples collected in the Ethylene Diamine Tetra Acetic Acid (EDTA) tubes. Polymerase chain reaction (PCR) technique was used for amplification of two viral genes *(UL55, UL97)* within host genes. Polymerase chain reaction results revealed that 89% and 77% of infertile women and 87% and 67% of breast cancer women were positive for these genes respectively.

Introduction

Human cytomegalovirus (from the Greek *cyto* "cell", and *megalo*,"large") is a member of the *Betaherpesvirinae* Subfamily, which belongs to the family *Herpesviridae* (Mocarski and Courcelle, 2001; Ryan and Ray, 2004; Staras *et al.*, 2006). The vast majority of people infected with HCMV do not exhibit any symptoms. Primary HCMV infections in immunocompetent individuals were mild or asymptomatic, or produce fever-like and mononucleosis-like symptoms (Cannon *et al.,* 2010). Like the other herpesviruses, after an initial primary infection HCMV establishes latency for the life of the host with periodic and spontaneous reactivation.

During the acute phase of infection, HCMV can infect a broad cell range within its host, including endothelial cells, epithelial cells, smooth muscle cells,

fibroblasts, neuronal cells, hepatocytes, trophoblasts, monocytes/macrophages and dendritic cells (DCs) (Sinzger *et al* ., 2008).

Materials and Methods

The current study was done in the Infertility Clinic of Kamal Al-Sammaraee Hospital and Al-Yarmouk teaching hospital and Radiation and Nuclear Medicine Hospital in Baghdad. The study was approved by the Babylon College of Medicine and informed consent was obtained from patients. The tested samples were included (330) whole blood samples from infected individuals with EDTA to perform molecular work that includes the detection of viral genes after DNA extraction using Favor Prep DNA extraction Mini Kit from *FavorGene* Biotechnologies (Taiwan) and *Geneaid* Genomic DNA Purification Kit from *Bioneer* (Korea), the DNA extracted according manufacturer instruction .In cases of women with infertility and cancer, 5 ml of blood was obtained at the admission time. Samples were prepared according to method of (Boeckh and Boivin, 1998; Koneman *et al*., 2006).

The primers used in this study were used for amplification of two genes which includes: 1- HCMV gB primers an *in vitro* nucleic acid amplification test for qualitative detection of *Human Cytomegalovirus* glycoprotein B gene (*UL55*). From Integrated DNA Technologies (USA), include these primers

F. GGTCTTCAAGGAACTCAGCAAGA
R. CGGCAATCGGTTTGTTGTAAA

The protocol was done as follows

Steps	Temperature and duration	
Initial denaturation	95°C for 7 min	
Denaturation	94°C for 30 sec	35 cycles
Annealing	63°C for 30 sec	
Extension	72°C for 30 sec	
Final Extension	72°C for 7 min	

2-HCMV phosphotransferase primer CMV is an *in vitro* nucleic acid amplification test for qualitative detection of *Human Cytomegalovirus* phosphotransferase (*UL97*) gene from *Synthesis gene* Technologies (China). Include this primer TATTAGGACAAGGCTGGTGGGCAC.

The protocol was done as follows

Steps	Temperature and duration	
Initial denaturation	95°C for 5 min	
Denaturation	94°C for 30 sec	35 cycles
Annealing	67°C for 40 sec	
Extension	72°C for 1 min	
Final Extension	72°C for 7 min	

Mastermix used a ready 50 µL PCR Mastermix (AccuPower® PCR Premix/Bioneer/Korea) was used for amplification for both genes. Template DNA (10 µL) from each sample and primers (5 µL from each) were added to each Mastermix tube. The mixture is then put in a shaker and spinner for 10 cycles for better mixing. After mixing, the Mastermix tubes were transferred to the thermocycler (Applied Bio system (Singapore) which is previously programmed with the above protocol according to the gene to be amplified.

1.5-2 gm of agarose (BioBasic/Canada) was dissolved in 100 ml of 1x Tris Borate EDTA (TBE) (BioBasic/Canada which used in the electrophoresis in addition to Ethidium bromide and 3000bp ladder. The statistical package for the social sciences (SPSS, version 14) was used for statistical analysis.

Results and Discussion

Molecular detection of HCMV DNA in infected groups by PCR technique

The molecular assays for HCMV diagnosis are considered to be beneficial and fast tools especially to susceptible groups such as transplant recipients and AIDS patients as well as infected children to determine differentiation between latent and active infection (Meguid *et al* ., 2004; Lashini *et al* ., 2011) . The PCR results were interpreted by the presence or absence of specific bands of amplified gene on 2% of agarose. The current study was conducted for amplification of HCMV gB (*UL55*), and phosphotransferase gene (Thymidine kinase, *UL97*) from whole blood specimens. The amplified products of these genes (*UL55, UL97*) were 72 and 800 bp respectively as in figure (1) and figure (2). The detection of HCMV DNA in whole blood became the standard method for monitoring the virus in immunosuppressed and unsuppressed patients. This result is in line with Mengelle *et al*., (2003) who showed that the detection of viral DNA in whole blood occurred more frequently than other specimens. The current results of PCR showed strong association with serostatus as in table (4-8) and (4-9) for both abortion and infertile women.

Human cytomegalovirus genes (*UL55* and *UL97*) were detected as in table (4-9). Infertile women reported for *UL55* and *UL97*, then there were 76(84%) and 72(79%) positive results respectively without significance. The current results are supported by Mengelle *et al*., (2003) who showed nearly same results when he was used whole blood. In regards to age the high prevalence of HCMV*UL55* was occurred at age 41-45years which reported 9(100%) followed by age 31-35 years that reported 24(89%), while the other gene was highly prevalent at age 31-35 which reported 26(96%) followed by age class 26 -30 which reported 29(91%) in case of miscarriages. The high prevalence of HCMV *UL55* occurred at age 26-30 years with 10(91%) prevalence, followed by age group 36-40 years with 19(90%) prevalence. For *UL97* gene, the high percentage was reported in age 31-35 years which is 18(90%) followed by 21-25 years which is 18(86%) without significance in case of infertile women. The current results were disagreed with Yasir, (2012) who showed low positivity of viral DNA in his selected groups. This may be due to the use of the other gene which was major immediate early gene (MIE) and it was detected at active replicating state and its expression restricted or absent during latency of the virus. However, these results were in line of Zhang *et al*., (2000) and (2010) who showed the high sensitivity of PCR for detection of HCMV which detect even latent non replicating virus in asymptomatic patients in young or old ages.

Several comparative studies have demonstrated that *gB* primer sets have high diagnostic sensitivity for HCMV infection (Barber *et al*., 1999; Distefano *et al*., 2004). The *gB* primer sets were used in the current study which amplify fragment of region that encodes the glycoprotein B,

Figure.1 PCR detection of HCMV in Ethidium bromide-stained agarose gel using specific primers gB gene of infected women showed 72 bp product and M.3000 bp marker, 1--6 positive results, 7 positive control.

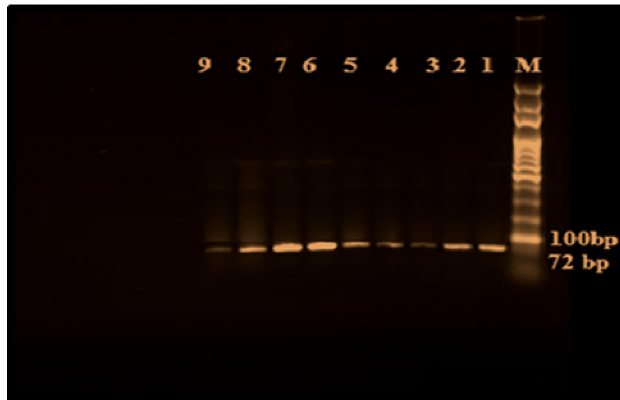

Figure.2 PCR detection of HCMV in Ethidium bromide-stained agarose gel using specific primer *UL97* gene of infected women showed 800 bp product and M.3000 bp marker, 2 negative result, 3-7 positive results, 1- positive control.

Table.1 Molecular detection of HCMV genes in infected groups by PCR technique

Groups of the study		UL55+	%	UL97+	%
Infertility	**98**	**81**	**89**	**75**	**77**
Breast cancer	**30**	**26**	**87**	**20**	**67**
Control	**50**	**41**	**82**	**33**	**66**

Table.2 Viral genes results within infertile age groups

Age groups	**Total No.**	**IgG +**		**Total IgG +**	**IgG -**		**Total IgG -**
		UL55+	***UL97+***		***UL55+***	***UL97+***	
16- 20	**8**	**6**	**5**	**7 88%**	**-**	**-**	**1 12%**
21-25	**22**	**16**	**18**	**21 95%**	**1**	**1**	**1 5%**
26-30	**12**	**10**	**7**	**11 92%**	**1**	**-**	**1 8%**
31-35	**21**	**16**	**18**	**20 95%**	**1**	**1**	**1 5%**
36-40	**22**	**19**	**15**	**21 95%**	**-**	**-**	**1 5%**
41-45	**13**	**9**	**9**	**11 85%**	**2**	**1**	**2 15%**
Total	**98**	**76 84%**	**72 79%**	**91 93%**	**5 71%**	**3 43%**	**7 7%**

this genomic region which considered as highly conservative of epitopes between clinical isolates (Shepp *et al*., 1996; Coyle *et al*., 2002). The highly prevalence of HCMV DNA in this study may be due to the detection of latently infected cells or extracellular DNA fragment in patient without active infection at the time of testing. This reason consistent with other reports which demonstrated same quantitative results (Roseff *et al*., 1993; Miller *et al*., 1994; Boeckh and Boivin, 1998).

Bolovan-Fritts *et al*., (1999) demonstrated that the analysis of peripheral blood monocytes from asymptomatic carriers showed that the latent HCMV genome was present in the cells as an episome. Glycoprotein B was a major target for neutralizing antibodies and an important component of recombinant vaccine that under trial, and there are many studies on genetic variability which depend upon glycoprotein B gene (Gandhoke *et al*., blocked p53 (cell cycle regulatory protein) thus inhibiting apoptosis and leading to 2013). Other gene that was studied is phosphotransferase gene (*UL97* region) of the viral genome which encoded the thymidine kinas, and genotyping assays that target for ganciclovir resistance gene. Ganciclovir resistance was mediated by mutation in this gene (Erice, 1999; Pass, 2001; Piiparinen, 2004).

For breast cancer women, the results showed that 26 (87%) were positive for HCMV *UL55*, while only 20 (67%) were positive for HCMV *UL97* DNA. The current result was supported by Taher *et al*., (2013) who showed that the HCMVDNA was abundantly detected in 100% of breast cancer specimens and 91% in the case of sentinel lymph node metastasis. Castillo and Kowalik, (2004) showed that the HCMV gene product was capable of transforming cells in *vitro* and the deregulation of cellular pathways considered to be relevant to the pathogenesis of carcinoma . Dupont *et al*., (2010) pointed that the HCMV was cell differentiation and proliferation.

Recently, Bender *et al*., (2009) showed that the virus acts as" hit and run" like mechanisms and may be present at some stage of cancer processing, this provides a useful indication that certain types of cancers are infected by HCMV and then trigger the oncogenic properties.

Harkins *et al*., (2010) who suggested that the persistent infection was occurred in high percentage of breast epithelium of normal adult females, but persistent viral protein expression correlated highly with tumorgenicity of breast cancer.

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