

## Detection of CMV Virus Using Two Methods: Real-Time-PCR and Cytochemical Staining in Immune-Compromised Individuals

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**ABSTRACT – BACKGROUND AND OBJECTIVES:** Human cytomegalovirus (HCMV) is a member of the family herpes viruses and early recognition of the importance of this virus is quite a lot of people are having is immunodeficiency, because of this, reason the extent of clinical protests infection to this virus in these patients, due reduced levels of their immune, the aim of this study was to detect the CMV virus quickly and check the accuracy of the two methods of Real-time PCR and cytochemical in immunocompromised people for HCMV defects. **METHODS:** 100 blood samples were collected from immunocompromised persons in Tehran. Real-time PCR and cytochemical staining two methods for the detection of HCMV was used. The first method is based on the Taq Man and second method was based is based on the characterization of the cell morphological identification. **RESULTS:** Those who PCR test results was positive, 40 were case (36%). Whereas, this number in of Cytochemical a total of 35 were cases (21 percent). It was also found that the maximum prevalence in the summer season (34%) and minimum its abundance belong to the autumn season (22%) is. However, a significant relationship was no observed between the prevalence of HCMV and the season. **CONCLUSION:** Results of this study showed that with greater accuracy Real-time PCR able to recognize HCMV infection in individuals is the immunocompromised. but cytochemical stains method can be used along with this method for the final confirmation and very increase the accuracy of diagnosis, Was also found that the highest rate of HCMV infection either latent or active form, is the related to the fall season, but this association were not significant.

**KEYWORDS:** Human Cytomegalovirus (CMV), Immunodeficiency, cytochemical staining, Real-time PCR.

## **Introduction**

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that rarely causes symptomatic disease in the immunocompetent host(1). Yet this virus has been at the frontier of virology since it was found to be involved in symptomatic diseases in immunocompromised individuals (2). Such clinical manifestations range from developmental abnormalities, mental retardation, deafness, mononucleosis, chorioretinitis, and fatal diseases like interstitial pneumonitis and disseminated HCMV infections (3). Cytomegalovirus (CMV), from herpes virus the family and are usually to be found in a lot of people. This virus is hidden in the body most people, but in a healthy host will remain passive (4). Those who associated with infection, the did not show no signs and symptoms, carriers of the CMV virus are called. Such infections can the esophagus, stomach, intestines, lung, eye, etc. will be affected. This is opportunistically of infections affect on more individuals that their immune system is weak(4) . Early detection of cytomegalovirus infection in the hosting body which have weakened immune systems, Along with the appropriate treatment (and Nutrient additives) order to prevention of serious complications which can a negative impact on have the prognosis is essential. HCMV infection in these individuals may even will result in death. Majority of people at the beginning of his life were placed exposed to cytomegalovirus (5). However, children and adults with symptoms of cytomegalovirus virus infection are rarely demonstrate. When the immune system becomes weak for various reasons CMV virus becomes active again the person affects with different intensity (6). A tool for the detection of CMV viral antigen in the blood, bone marrow and organ transplant are in patients. The polymerase chain reaction (PCR) and highly sensitive method for the identification of is the HCMV (7). HCMV infection in immunocompromised individuals as well as to confirm the diagnosis of a standard method in this article be raised for the first time, called cytochemical stains. It is based on morphological characteristics of the are cells (8).

## **Materials and methods**

### ***Sampling and DNA extracted***

100 blood samples from 2012 July until 2013 May in immunocompromised persons were collected. Of this number, 50 were male and 50 were female. For the DNA extracted were treated accordance with Qiagen mini DNA #51304 Germany kits protocol.

### ***RT-PCR and perform confirmatory testing Cytochemical***

HCMV detection of the two quantitative polymerase chain reaction (Real-time PCR) and identify morphological characteristics of cells and cell changes from method study cytochemical stains was used. Process to perform Real-time PCR which based on the probes (Taq Man®-MGB probe) was based. Accordance with protocol Q-HCMV Real Time Complete Kit was operated. As well as for the detection of HCMV, cytochemical staining kit Sigma, Germany was used which based on peripheral blood cells is based.

### ***cytochemical staining method***

Of the each Peripheral blood sample, 11 slides were prepared and were coded by diamond pen. Then by fixator solution we do fixing action. To study the Leukocyte characteristics, we stain 1 slide toluidine blue staining method. examined is preliminary until according to the morphology of the white blood cells, white blood cells the tonality listed in the population examined is preliminary until as if there is a difference samples collection method so that study white blood cells is inefficient be clear. for the differential diagnosis of adult cells based on the classical identification of white blood cells, cytochemical stain naphthol AS-D Klrvastat esterase, alpha-naphthyl acetate esterase, acid phosphatase, periodic acid-Schiff using the of commercial books manufactured by Sigma-Aldrich was done (9) .

### ***Study of cells appearance variations***

All the cosmetic changes of cells including cells quality, inflammatory changes, in toluidine blue staining were studied.

### ***Study of leukocyte cells***

To check leukocyte cells are was used from cytochemical stain, and the samples examined under a microscope. Eosinophils by periodic acid-Schiff stain and acid phosphatase were observed under optical microscope with 100 × lens.to see the basophils with optical microscope of periodic acid-Schiff stain and toluidine blue was used. In toluidine blue and PAS staining of mast cells and basophils and eosinophils are visible. Monocytes by Alpha-naphthyl acetate esterase staining naphthol AS-D chloroastate esterase are indicated. Lymphocytes also by A- naphthyl acetate esterase and naphthol AS-D Klrvastat esterase and acid phosphatase stains are recognized. that all positive results and negative tests for the virus can be expressed as a percentage of results (10).

### ***Toluidine blue staining***

Slides with a solution of 1 volume of ethanol, 1 volume of methanol, and 3 volumes of acetone fixed, after transfer to the laboratory slides ready to put on a tray coloring, and Toluidine blue on the grapefruit throw, after 15 to 20 minutes offer with water slides, after drying the slides can be viewed with the microscope.

### ***Naphthol AS-D chloroacetate esterase staining***

In this method, proven solution by mixing 18 ml of citrate solution, and 27ml of acetone and methanol (5ml) was built, and the slides were placed in a minute, the solution to be prove, then washed with deionized water, and then a solution of 6/3 Trizymal by dissolving one part of concentrated buffer 6/3 Trizymal to9 parts of deionized water was prepared, and added to the a salt capsule V fast Corrientes and then 2 ml naphthol AS-D chloroacetate esterase solution, added and mixed for 30-15 seconds. All slides were prepared solution for 5 min. were washed with deionized water for 3 minutes. Naphthol AS-Dchloroacetate solution by dissolving a capsule Naphthol AS-D chloroacetate in 2 ml dimethyl formaldehyde obtained.painting background slides for 5 to 10 minutes in a solution of haematoxylin acid and then rinsed with running water and dried in the open air, target cells by light microscopy to identify the specific and measurement were performed.

**Alpha naphthalen propanoic acetate esterase staining**

In this method, proven solution by mixing 18ml of citrate solution and 27ml of acetone and methanol (5ml) was built, and the slides were placed in one minute, the solution to be done was to prove, then rinsed with deionized water, then PH=7/6 Trizymal solution, by solving a 7/6 trizymal dense buffer to 9 parts of deionized water was prepared, and acapsule RR salt, and then add 2ml naphthalen propanoic acetate was mixed for 20-15 seconds. All slides were prepared in the solution for 30 minutes and then washed with deionized water for 3 minutes. naphthalen propanoic acetate solution by dissolving acapsulen aphthalen propanoic acetate in 2 ml of mono methyle ther-Glycol obtained, Painting background slides for 5-10 minutes in a solution of haematoxylin and then washed in running water were dried in the open air. Target cells by light microscopy to identify species and micrometer measurements were examined and photographed.

**Acid phosphatase staining**

In this method, proven solution by mixing 25 ml of citrate, 65 ml of acetone and 8 ml of 37% formaldehyde was made, and the slides were immersed for 3 seconds in this solution to be fixed, then washed with deionized water. Then the two tubes 5.0 ml Fast Garnet GBC solution and 5.0 ml of sodium nitrite solution was poured and was mixed for 30seconds. In a large glass jar, 45 ml of deionized water One ml Fast Garnet GBD solution that the Previously prepared 5.0 ml Naphthol AS-BI phosphate solution two ml of acetate was added, and the slides were placed for one hour in a Jar, after this time, washed with deionized water, and for coloring the background for two minutes in a solution of haematoxylin were placed. After washing with running water, dried in the open air, target cells by light microscopy to identify specific and micrometer measurements were examined and photographed.

**Periodic acid-Schiff staining**

In this method, fixation solution by mixing 5 ml of formaldehyde with 45 ml of ethanol 95° C was produced, the slides were exposed for one minute in a solution to the fixing operation to be performed, the slides were washed in running water for one minute and then placed 5 minutes in Periodic acid solution, the slides were washed well with distilled water. 15 minutes were Schiff solution, next 5 min washing with running water, and in painting background were 90s in a solution of haematoxylin, slides were washed in running water must be drained, target cells by light microscopy to identify species and micrometer measurements were examined and photographed.

**Alkaline phosphatase**

- 1 -The first 45 ml of distilled water to bring the temperature 18 to 26 ° C.
- 2- Then we prepared diazonium salt solution in which 1ml of sodium nitrite are added to 1ml of alkaline FRV.
- 3- And then the salt solution prepared in distilled water which had been prepared in the first step are added.
- 4- Then 1 ml Naphthol AS-bitothedia zonium prepared salt solution adding, and it into a glass jar poured, and well in incorporate.
- 5- The sample is poured into jars and we wait 30 seconds and then the samples were washed with distilled water for 45 minutes, and then bring to the slides well dry, after all the samples in the baseline alkaline solution for 15 minutes, so stay from direct light, to get a good color, because these colors are sensitive to light and the light loses its enzymatic activity and the disabled, after 15 min of incubation for 2 minutes were well washed with distilled water and bring to the dry slides, and then microscopic we evaluate the samples.

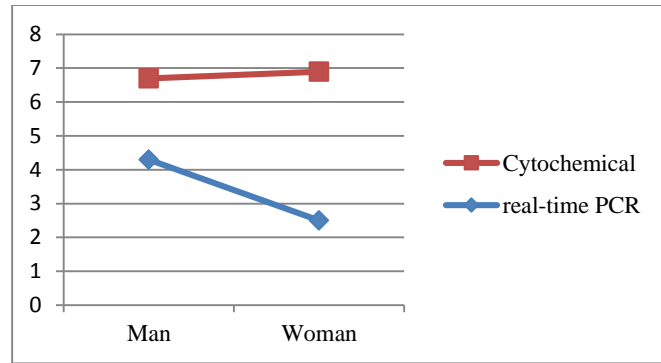
**Results**

In this study, for accurate identification of human cytomegalovirus (HCMV) in immunocompromised personae of Real-time PCR method and stained Cytochemical was used. The number of people who them Real-time PCR test was reported positive, 40 (36%), was patients. which included were 20 men (50%) and 20 women (50%), (Table 1). In the Cytochemical method, a total of was positive 35 (21%) samples. of the 35 cases, were 17 males (2/55) and 18 women (8/44%), (Figure 1).

**Table 1:** Carefully identification of human cytomegalovirus in immunocompromised persons based on the two Real-time PCR and Cytochemical method

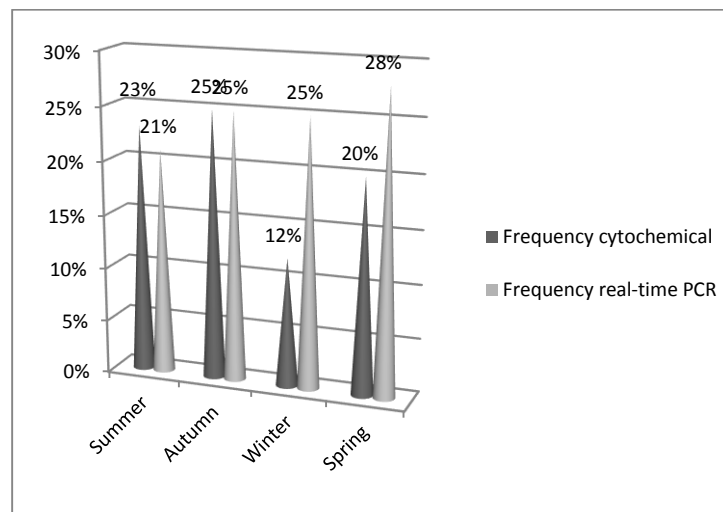
PCR results			
pp65 antigen detection	Positive (69)	Negative (146)	Inconclusive (12)
Positive (12)	10	1	1
Negative (192)	52	133	7
Inconclusive (23)	7	12	4

Cytochemical results			
pp65 antigen detection	Positive (69)	Negative (146)	Inconclusive (12)
Positive (12)	5	0/5	0/5
Negative (192)	26	51	2.5
Inconclusive (23)	2.5	6	2

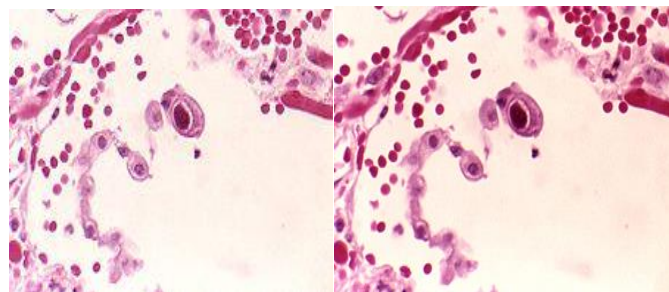


**Figure 1:** amount identification of human cytomegalovirus in immunocompromised persons based on RT-PCR and Cytochemical method.

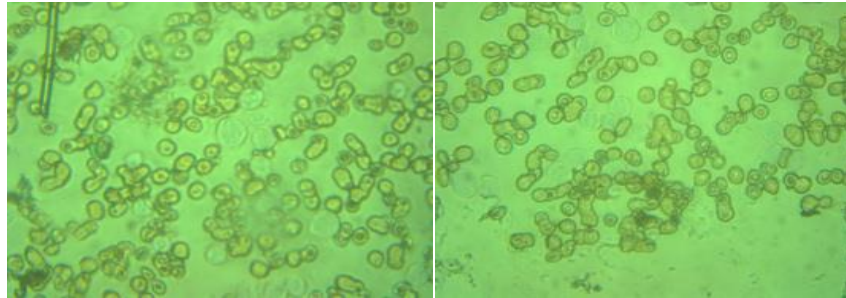
Evaluate the frequency of HCMV in different seasons of showed that the highest prevalence of this virus is in the summer season, Therefore, this frequency based on the two Real-time PCR and Cytochemical method was respectively 34% and 22%, . However, the lowest prevalence of this virus for Real-time PCR method belongs to the autumn and belongs to the was summer for cytochemical method. Evaluate the frequency of active infection also indicated that the maximum frequency of this type of infection in summer season (34%) and minimum frequency belongs to the autumn season (22%). Then the frequency of latent infection and active in between men and women in different seasons of were studied. Results of this study showed that prevalence of active and latent infection in the autumn in women twice as often as men. Whereas men in summer season to 7 times higher than women to indicate active and latent infection (Figure 2).



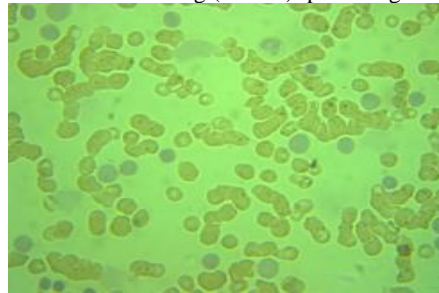
**Figure 2-**examine the impact of season on the prevalence of cytomegalovirus



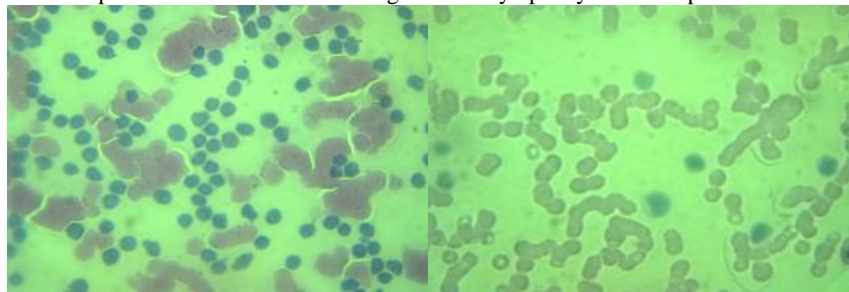
**Figure 3-** morphological characterization of cytomegalovirus infection by cytochemical stains (alkaline phosphatase, periodic acid-Schiff).



**Figure 4.** Alpha-naphthalenepropanoic acetate esterase staining (ANAE): positive granules in B and T – lymphocytes.



**Figure 5.** Acid phosphatase (Aph): positive reaction in thrombocytes, lymphocytes, neutrophils and morphological characteristics Prolymphocytes and T - lymphocytes shows, Studies show that these colors in blast cells in the region show a positive reaction from the Golgi. T-CLL lymphocytes is also positive.



**Figure 6.** Periodic acid shift (PAS):L: two blast cells with PAS have been extremely positive, one round and the other against the background of positive cytoplasmic granules

### Discussion and conclusion

In immunocompromised persons, the most severe clinical symptoms of infection with HCMV were observed(11).Timely and accurate detection of CMV in individuals who are immunocompromised is important, Because, HCMV is one of the main causes mortality in this is patients (11).In this study, for identification HCMV in 100personimmunocompromised, were used from both Real-time PCR and CytocheMICAL methods. The in Real-time PCR method 40 people were positive and in the cytochemicalmethodwere 35 people. This may represents higher precision of PCR method is compared to the cytochemical and these results are consistent with previous studies(12).Real-time PCR also has the additional advantages compared to the CytocheMICAL which include being simpler, higher speed and more ability of this method to study the a larger number of samples is simultaneously (13).The remarkable thing these two methods how to identification of is the virus. which in this case the just virus were detection and there is no distinction between active and latent infection (14).If instead of qualitative PCR from quantitative PCR (Real-time PCR) is used. But the cytochemical method is based upon the morphological characteristics identification of the cells. this method can also will help to confirm the diagnosis of virus, and also if in patients referred suspected of having the virus in first order to be examined by cytochemical method can to give us a prognosis about the disease and after the diagnosis is confirmed by RT-PCR method (15).actually this method cannot detect latent infection. On the other hand know that the basis of identification of HCMV in Cytochemicalmethod based on the identification of prion particles on the surface of leukocytes (cells, CD8 + T lymphocyte is a type of (16).So it can be concluded that patients who likely to positive Real time-PCR test but negative Cytochemicaltests, Exposed to reduce leukocytes (leukopenia) located, And recommended that these patients be placed undergoing clinical complete care. The pattern obtained in this study direction evaluate the prevalence of HCMV infection during different seasons showed that the highest active infection were in summer and lowest in autumn (Table 2).However, no significant associations between HCMV infection and season may be due to small sample size was not observed. This is while studies Singh and his colleagues in the United States of America are meaningful (p = 0.09) showed that HCMV infection in immunocompromised persons the highest frequency is in autumn (17).

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