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The prevalence of active CMV infection in renal transplant recipients using PCR and indirect immunofluorescence assay

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ABSTRACT

Background and Objectives: Cytomegalovirus (CMV) is one of the most important pathogens which its presence has been studied in transplant recipients. The virus could cause significant illness and mortality. The prevalence of CMV infection in transplant recipients has been reported to vary from one country to another country. Indirect immunofluorescence method indicates viral replication and active infection, and PCR method, illustrates the presence of virus. The aim of this study was to determine the prevalence of active CMV infection in kidney transplant recipients with PCR and indirect immunofluorescence methods and also its incidence according to season, sex and age variables.
Methods: Samples of volunteer patients, sent from all kidney transplant centers throughout the country, from 2006 to 2007, to Gholhak laboratory ,were studied. The study performed by PCR and indirect immunofluorescence assay (pp65 antigenemia). Results were analyzed by Chi-square test and logistic Regression.
Results:Totally923 samples were analyzed. CMV infection, using Antigenemia pp65 and PCR method, was found in 71(7.7 %) and 323 (35%)of thecases . There was no significant association between the positive rate of CMV presence, and age and sex (P> 0.05). There was a significant relation between presence of CMV and season of doing the test (p = 0.01). The most positive results in both methods, was in the spring.
Conclusion: The results of the PCR method is more than the results using the pp65 Antigenemia method. None of the methods show any relations with age and sex. Infection has a significant increase in spring.
KEYWORDS: Cytomegalovirus, Epidemiology, antigenemia, $pp_{65,}$PCR, kidney transplant.

INTRODUCTION

Human Cytomegalovirus (CMV) infection has been observed all-around the world. Prominent effects of the infection in transplant recipients, varies from clinical demonstrations of acute CMV disease to damage to the transplanted organ and transplant rejection (Mocarskiet al, 2007).

Despite using antiviral drugs and many attempts that are done to prevent from this infection, infection with the virus is still a major cause of morbidity and mortality after bone marrow and kidney transplantation (Erice, 1999). Studies have shown that individuals with seropositive CMV, are in the risk of infection or CMV recurrence (Schulenburg et al, 2001). CMV disease incidence often is seen in a period of time, about 28 and 72 days after transplantation, and could affect several organs, including lung and colon. Despite great developments in this field (production of new antiviral drugs, combination therapy), rates of mortality from pneumonia caused by CMV is still high (ibid).

Considering that the major cause of Cytomegalovirus disease is infection with the CMV virus, early diagnosing in order to prevent disease progression is recommended. A standard classic method for the diagnosis of CMV infection in transplant recipients is Antigenemiapp_{65} technique.. In this method, the specific ant given for CMV, which is expressed by cells in the early stages after the infection, is being detected (Forman and Zaia, 1994), however, this method, sometimes, in a small number of patients, due to the low level of antigen expression in white blood cells, shows false negative results.

However, the sensitivity of PCR technology, which is used in recent decades, for the diagnosis of CMV virus DNA, in blood samples, is 100%. The PCR method generally is more sensitive than pp_{65} method, for virus detection, but is unable to distinguish active and hidden infection. While the pp65 test, is used for distinction between active and latent infection. The aim of this study is to determine the prevalence of CMV active infection, in kidney transplant recipients, with the two methods of PCR and PP_{65} antigen methods. On the other hand, evaluation of correlation between the infection prevalence with season, age and sex was of sub-goals of the study.

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MATERIALS AND METHODS

This study was a descriptive and prospective type. Samples between the years 1385 to 1386, were collected, from Gholhak laboratory, as one of the most important transplant experimental centers in Tehran. For this purpose, two 4-mL tubes containing EDTA, were collected from blood sample. Then Plasma was centrifuged at 500xg for ten minutes and then stored at -20 ° C.

Method:

The pp_{65}antigenemiamethod was carried outas a common diagnostic method,byusingBrite^{TM}Turbu (IQ Products CMV Groningen, Netherlands)kit.pp_{65} antigen detection in peripheral blood cells, helps diagnosis of acute or reactivated CMV infection . In this method a compound containing 2Monoclonal antibodies (C10/C11) is used against pp_{65} antigen with indirect immunofluorescence method.

CMV virus DNA extraction and PCR:

DNA was extracted from 200 ml of peripheral blood mononuclear cells by Phenol –chloroform method and was kept in tubes containing 50ml of buffer. Primers designed by the Gene runner program (version 3.o5, Hastingssottunre Inc) for a protected area of pp_{65} antigen include:

Leading5- TCG CGC CCG AAG AGG 3

Follower5- CGG CCG GAT TGT GGA TT3

PCR method was performed in total reaction volume,25µL.The reaction tube contains 2.5µL of PCR buffer(ferment as), dNTP(10mM) 0.5µL,1µL of 5 u/ µL DNA taq polymerase and 0.75 µL (50mM) $MgCl_2$.

5µL of extracted sample, containing 0.5µg and 0.5µL of each of the leading and following primers were mixed. Proliferation procedures including primary denaturation in 94° C for 6 minutes, then 35 cycle, include50 Seconds in 94 ° C, 40Second in 57 ° C , 50 Seconds in 72° C ,and at the end 7 minutes at 72° C.

Statistical analysis:

The obtained Information(data) was entered into the SPSS statistical program. In order to data analysis, the Chi-square test (Chi-2) and the Logistic Regression were used. P-value was less than 5 Percent with confidence interval%95 ,considered as the limit of significance.

RESULTS

923 samples containing 569(61.6%) of male subjects and 354(38.4%) female samples, were studied. 323 (35%) and 71(7.7%) sample, respectively, were positive by using PCR and PP_{65} methods. there was no significant relationship between age, sex and results of none of the PCR and PP_{65}tests (p>0.05).The most positive results with PCR method (50%) was seen during spring (p = 0.01). The most cases of active infection (PP_{65} positive), was also observed in the spring (60%) (p = 0.01). Percent of positive results, with PCR and PP_{65} methods, presented in table1.

Table 1, shows the percentage of PCR and antigenemia tests results, during the four seasons of the year. Chi-square test indicated the relation between the season and the PCR and also antigenemia test results (p = 0.0) .

Table 1. Results of Antigenemia and PCR methods separately in Season

Test	result	spring	summer	autumn	winter	total
PCR	positive	50%	15%	18%	17%	100%
pp_{65}	positive	60%	20%	2.9%	17.1%	100%

Table 2. Results of Antigenemia and PCR methods separately in Sex

sex	result	PCR	PP_{65}
man	negative	63%	93%
	positive	37%	7%
woman	negative	67%	91%
	positive	33%	9%

Table 2, shows PCR and Antigenemia tests for both sexes. Chi-square test indicated no correlation between PCR experiments, sex (P = 0.31) and Antigenemia (P = 0.638).

The Logistic regression was used to investigate the relationship between age with PCR and antigenemia tests. Age coefficient in Logistic regression equation was β=0.005With p = 0.28 and β=0.013with p = 0.15, respectively. Thus, reflects the lack of correlation between age with PCR and antgenemia tests.

DISCUSSION

Human Cytomegalovirus is one of the most common infections, in human populations, which in healthy people is without clinical symptoms. But after the primary infection, remains in the body, forever. Acute infection appears in immunocompromised patients and in transplant recipients (Vahid et al, 2005). Despite the appropriate antiviral drugs (Ganciclovir, Foscarent), the CMV infection, still remains as an important cause of disease and mortality in transplant patients, that is often because of delayed diagnosis and appropriate treatment .

Diagnosis with Cell culture and serological methods, is usually time consuming and sometimes do not have enough sensitivity. The presence of pp_{65}antigen. indicates viral replication and active infection . Whereas positive results with PCR method only indicates the presence of virus, which is not able to distinguish between active and latent infection. Since a large proportion of the population are infected with the CMV virus ,In screening the patients for assessment the risk of disseminated infection,usingpp_{65} antigenemia test seems appropriate and necessary(8).In this study, the incidence of CMV infection by PCR and PP_{65}Antigenemia methods, was calculated 35 % and 7.7 % , respectively . The results have close agreement with the other studies (Rao, 2002).

Among the studied variables (age, sex and season), significant relationship was observed, only between the positive CMV rate and the season. Therefore, most of the positive results were related to spring. One of the most problems of detecting CMV infection diagnosis with Serological methods is delay in diagnosis time. In these cases, Antigenemiatest helps for early diagnosis (1). In a study was shown that antigenemia test results in 2kidney transplant recipient patients, 8 And23days before death due to CMV infection, was negative (Rao et al, 2000).

Clinical doubt to CMV disease and proceed for early diagnosis and treatment of the disease in kidney transplant recipients, especially in the first 6month after transplantation, has the undeniable importance (Mocarskiet al, 2007).

Data analysis showed that men and women in age groups36-45and 46-55Years, respectively, had more negative Antigenemia results. On the other hand, men in the age group 55-65andwomen aged 36-45, showed the most positive results in this experiment.

In the PCR test, men in 55-65age group and women in 36-45 age group, show more CMV infection. In contrast, with the experiment, men aged 26-35and women aged 36-45, have more negative results. However, the differences between the data are not as significant to express statistical relationship. Indeed, there is no relationship between the age and sex with the test results.

The results suggest a relationship between the positive results rate of PCR method, in the spring. In one study it was shown that liver transplantation in the fall season is an important risk factor for CMV (Robinson et al, 1999) .In another study Nita et al., suggest the relationship between CMV disease incidence and autumn. So that patients receive transplant in autumn, the incidence of CMV incidence is higher in them (Nina et al, 2001). However, in other reports about whether the association between CMV infection and the seasons, results are varied (Robinson et al, 2000). Studies show that, a number of diseases have a seasonal pattern. This pattern could be considered, as an important factor to identify the etiology of the disease. One of the other necessities in review the relationship between an infection and the season and to determine seasonal pattern, especially in the case of viruses such as CMV, is Prophylax is associated with the season. However, further studies are needed on this subject.

Conclusion:

Our study, similar to other studies, in addition to indicate seasonal relationship between kidney transplant receive and CMV infection, with sensitive methods, reflects the fact that timely and exact diagnosis the active form of infection, as well as, investigation the presence of latent infection, and the beginning of its activity in the time of transplantation, is one of the requirements of diagnosis. This subject could be helpful in delivering treatment strategies.

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