Bangladesh Med Res Counc Bull 2011; 37: 28-33

Higher prevalence of Cytomegalovius pp65 antigenemia associated with lower CD4$^+$ T lymphocyte count

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Abstract

Sixty seven immunocompromised patients were studied prospectively to observe the association of Cytomegalovirus (CMV) pp65 antigenemia with CD4$^+$ T-lymphocyte count, a marker of cellular immunity. This study was conducted in three different groups of immunocompromised patients including HIV infected patients, patients with haematological malignancy and kidney transplanted patients. Result of the study indicate that proportion of severely immunocompromised patients having lower cellular immunity (≤200/µl) in all the three groups were comparable and ranged from 44% to 50%. The study also indicated that high prevalence of CMV pp65 antigenemia was associated with lower level of cell mediated immunity (≤200/µl). pp65 antigen was detected in 40% of patients with low immunity in contrast with 20% and 22.72% in the patient with intermediate immunity and in group without gross immune deficiency respectively. Lower level of cellular immunity was also associated with high level of CMV pp65 antigen. This was indicated by 75% patients with low immunity having high level of CMV pp65 antigen. It may perhaps be concluded that CMV infection occurred in a higher rate in immunocompromised patients and this is highly associated with the lower immune status of the immunocompromised patients as well as the level of CMV pp65 is higher in the patients with lower cellular immunity. This perhaps indicate that CMV infection is more severe in patients with low cellular immune response.

Introduction

Cytomegalovirus (CMV) infection is a major cause of disease in immunocompromised individuals including Acquired Immunodeficiency Syndrome (AIDS) patients and allograft transplant recipients[1-3]. The most severe infections are seen in recipients of allogeneic bone marrow transplants and in AIDS patients with very low CD4$^+$ T lymphocytes counts[4,5]. CMV diseases are more frequent in solid organ transplant patients and in patients receiving immunosuppressive chemotherapy for cancer or collagen vascular disease[6-8]. The clinical effects of CMV infection include infectious disease syndromes such as prolonged fevers, pneumonia, hepatitis, colitis and chronically progressive chorioretinitis[3,6]. CMV may predispose the patient to life threatening super infection with a variety of microbial agents, including gram negative bacilli, L. monocytogens, P. carinii, and fungi i.e. Aspergillous species, Cryptococcus neoformans, and Candida species[6,9]. CMV may initiate a process that can result in allograft injury in case of transplant recipients[10,11].

Pathogenesis of CMV disease is directly linked to the immune status of the host. Innate and adaptive immune mechanisms control acute virus infection, maintain latent infection, and modulate virus during reactivation. In the steady state of a latent CMV infection, the immune response is dominated by CMV-specific CD4$^+$ T cells. In single individuals, these CMV-specific CD4$^+$ T cells can reach extremely high frequencies and may play an important role in maintaining the state of antiviral immunity[12]. Although it is likely that immune control of viral infection, in general, is dependent on combined effect of intact cellular and humoral immunity, it appears that recovery from CMV infection is dependent to an

exceptional degree on the function of the cellular immune system. Patients who have depressed levels of mononuclear cell cytolytic activity against CMV-infected targets and who do not have any detectable CMV-specific cytotoxic T cells in the peripheral blood during infection usually die of overwhelming infection[5]. A loss of CMV-specific $CD4^+$ T cells in the first months after transplantation of CMV seropositive renal transplant recipients not only correlates with an uncontrolled viral replication but also with an increased incidence of CMV-related diseases[13]. Moreover, a progressive functional impairment[14] and a decrease in the level of CMV specific $CD4^+$ T cells correlate with impaired CMV control in patients after renal, heart and lung transplantation[15].

Fatal CMV infections and the absence of detectable CMV-specific cytotoxic T cells in peripheral blood is also observed among individuals with AIDS[16,17]. Moreover, the majority of patients who are studied repeatedly over a 2-6 months period exhibit a progressive decline in these immune functions: this decline closely parallels the progressive decrease in the number of helper/inducer $CD4^+$ T lymphocytes in peripheral blood. Thus, knowledge of absolute numbers of peripheral blood $CD4^+$ T lymphocytes provides a good estimation of the integrity of anti-CMV defense mechanisms in immunocompromised patients. Thus, this study was carried out to asses the immune status of the three different subsets of immunocompromised patients and also to observe the association of CMV infection with the $CD4^+$ T lymphocyte count.

Materials and Methods

This prospective study was conducted among three different groups of 67 immunocompromised patients to see the association of CMV pp65 antigenemia with their $CD4^+$ T lymphocyte count, a marker of cellular immunity. Three different groups of immunocompromised patients consisting of 30 HIV infected patients, 25 patients with malignant disorder of blood and 12 patients with renal transplantation. HIV infected patients referred from Ashar Alo Society and CARE Bangladesh were enrolled in the study. Kidney transplanted patients were recruited from the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU). They were recruited after one month of transplantation. Patients with malignant disorder of blood who had received chemotherapy were enrolled from the Department of Haematology and Oncology, BSMMU. All these patients from the three groups were included in the study as they were considered as immunocompromised. HIV infection is universally accepted as a cause of immunosuppression, patients with haematological malignancy were under immunosuppressive chemotherapy and renal transplant patients are deliberately immunosuppressed to maintain the transplanted kidney. The age of the participants ranged from 7 months to 60 years and were from both sexes. Fifty seven patients were more than 20 years of age, whereas only 12 patients of acute lymhoblastic leukaemia, a malignant disorder of blood were below the age of twenty years. Patients were not enrolled according to age and sex but rather on their availability.

The $CD4^+$ T lymphocytes were counted by flow cytometry to asses the immune status of 67 immunocompromised patients. CMV pp65 antigen was tested prospectively for 5-7 months at an interval of 4 weeks. Since the incubation period of CMV infection is 4-8 weeks, the patients were tested for pp65 antigen every 4 weeks to detect the new infection except a few who failed to comply with the date of visit. CMV pp65 antigen was tested by employing indirect immunofuorescence test. Blood samples were collected using aseptic venipuncture technique. The samples were labeled and case number was recorded on the clinical data sheet immediately. Informed written consent was taken from each patients and from the guardian of the patients who were minors. The laboratory works were carried out at the Department of Virology, BSMMU and the Armed Forced Institute of Pathology (AFIP), Dhaka during the period of January to December 2008.

CD4 lymphocyte cell counts were performed with a commercially available kit (Partec NoLyse-NoWash CD4, Partec GmbH, Munster Germany, code No. BDA004CJ/05-8500) following the manufacturer instructions. CD4-PE fluorescence can be analyzed on Partec CyFlow flow cytometer with an excitation light source of 532 nm (green solid state laser). On the basis of

their CD4 cell count the immunocompromised patients were classified into 3 categories. These are CD4 count ≤200, CD4 count 201-350 and CD4 count >350 per µl of blood. This classification of CD4 counts are used for HIV infected patients which indicate low immunity, intermediate immunity and without gross immune deficiency respectively. On the basis of this classification antiretroviral therapy is administered to HIV positive patients (WHO, ART guide line, revised in 2003, published in 2004).

The CMV pp65 antigenemia assay was performed with a commercially available kit (CMV–vue kit, Diasorin Inc. Stilwater, Minn. U.S.A. Cat. No.32500) according to the manufacturer's instructions. Results were expressed quantitatively as the number of CMV antigen-positive cells per 200,000 cells examined. Fewer than 10 positive cells were considered as low positive result, 10 to 49 positive cells as intermediate result, and 50 or more positive cells was considered as highly positive result[18,19].

Statistical analyses

The data obtained from the study were entered into SPSS-10.0 for windows and analyzed. Test of significance was estimated by using the statistical methods. Wilcoxon signed rank test and Kruskal-Wallis test were used for analysis. Probability value <0.05 was considered significant.

Results

Patients were between the age of 7 months to 60 years. Mean age was 28.76 years. 5 cases were within the age range of 0-10 years. Of them only one case was pp65 antigen positive (figure-1); 3 were positive among 7 cases of 11-20 yrs age range. 7 cases among 25, 6 cases among 22, 3 cases among 6 were positive in the age range of 21-30, 31-40, 41-50 respectively. There was no pp65 antigen positive cases among 2 of 51-60 years age range. Figure-2 shows that 40% were pp65 antigen positive among 29 female whereas 60% were positive among 38 enrolled male cases.

Table-I shows that among the 67 study patients, 31 (46.27%) had ≤200/µl $CD4^+$ T lymphocyte count and 16 (23.88%) and 20 (29.86%) had CD4 cell count of 201-350 and >350 respectively. Of the 30 HIV infected patients, 14 (46.66%) had ≤200/µl CD4 T lymphocyte count, and among the kidney transplanted patients and patients with malignant disorder of blood 06 (50%) and 11 (44%) were with ≤200/µl CD4 cell count respectively. This result indicates that statistically a significant no of patients from the different groups had a low $CD4^+$ T lymphocyte count (≤200/µl) indicating low immunity (Wilcoxon signed rank test, p=.009). Proportion of severely immunocompromised patients from all the 3 groups were comparable ranging from 44% to 50% (Kruskal-Wallis test, p=.7) indicating that there was no significant difference between the groups.

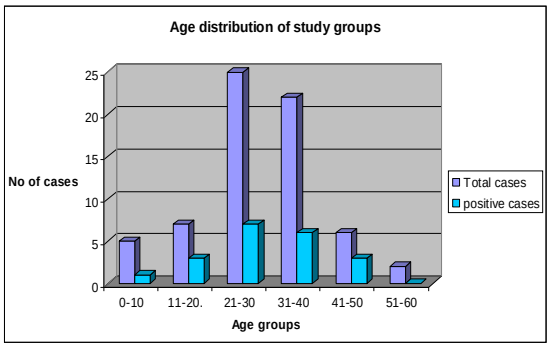

Figure-1: Age distribution of study groups

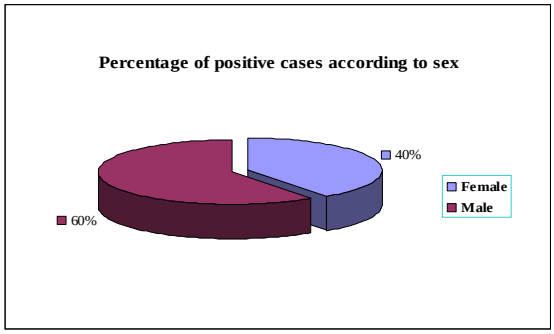

Figure-2: Percentage of positive cases according to sex

Table I: $CD4^+$ T lymphocyte count among the immunocompromised patients group

Patients group	$CD4^+$ T Lymphocyte			Total
	≤200	201-350	>350	
HIV infected patients	14 (46.7%)	09 (30.0%)	07 (23.3%)	30
Kidney transplanted patients	6 (50.0%)	3 (25.0%)	3 (25.0%)	12
Patients with malignant disorder of blood	11 (44.0%)	04 (16.0%)	10 (40.0%)	25
Total	31(46.3%)	16 (23.9)	20 (29.8%)	67

Table II shows that pp65 antigen was detected with higher frequency in patients with ≤200 $CD4^+$ T cell count. Thus, pp65 antigen was detected in 12 (40%) out of the 30 patients with CD4 cell count ≤200/µl, 3(20%) out of 15 patients and 5 (22.72%) out of 22 patients with CD4 cell count 201-350/µl and >350/µl respectively. Proportion of pp65 positive cases in the ≤200 CD4 cell count group was statistically highly significant (p<0.001), indicating that CMV primary infection and reactivation are more common in individuals with lower level of cell mediated immunity.

Table III shows association between level of immunity and of pp65 antigen. Among 12 pp65 antigen positive cases with low immunity or ≤200/µl CD4 cell count, 9 (75%) had high level of antigenemia and 3 (25%) had intermediate level of antigenemia. Of the 3 pp65 antigen positive cases with 201-350 CD4 cell count, 2 (66.67%) had intermediate level of antigenemia, while 1 (33.33%) had low level of antigenemia. Out of 5 pp65 antigen positive cases with >350 CD4 cell count, 4 (80%) had intermediate level of antigenemia and 1 (20%) had high level of antigenemia. These results indicate that low CD4 (≤200/µl) cell count was significantly associated with high level of antigenemia (P=0.011).

Table II: Detection of pp65 antigen in immunocompromised patients with different level of CD4 counts

CD4 groups	pp65 antigen Detected	Not detected	Total
≤200	12 (40%)	18 (60%)	30 (100%)
201-350	3 (20%)	12 (80%)	15 (100%)
>350	5(22.7%)	17 (77.3%)	22 (100%)
Total	20	47	67

Wilcoxon signed ranks test was done (p<0.001).

Table III: Association between level of pp65 antigen with $CD4^+$ T cells

$CD4^+$ T cells	CMV pp65 antigen			Total
	Low-level antigenemia	Intermediate level antigenemia	High level antigenemia	
≤200	0 (00%)	3 (25%)	9 (75%)	12 (100%)
201-350	1 (25%)	2 (75%)	0 (00%)	3 (100%)
>350	0 (00%)	4 (80%)	1 (20%)	5 (100%)
Total	1	9	10	20

Wilcoxon signed ranks test was done (p=0.011).

Discussion

The occurrence of CMV disease during immunosuppressive treatments and in AIDS patients demonstrate the importance of cellular immunity in the control of CMV infection. The profound immunodeficiency associated with transplantation, HIV infection is permissive to uncontrolled CMV replication results in the development of end organ damage[15-17]. In this study HIV infected patients, patients with haematological malignancy and kidney transplanted patients were exhibit low CD4 T cell count (≤200/µl) indicating low cellular immunity irrespective of the cause of immunosuppression (Table-I). Thus among the 67 immunocompromised patients from three different groups, 31 (46.27%) had ≤200/µl CD4 T lymphocyte count.

HIV infection results in damage to cellular responses[20]. Most antiretroviral (ARV)–untreated patients eventually experience increased viral replication, accelerated $CD4^+$ T cell depletion, and disease progression. Although multiple mechanisms may account for the failure of the cellular immune system to control viral replication, virus-mediated destruction and/or dysregulation of HIV-1–specific $CD4^+$ T cells is probably a primary defect that leads to the loss of virus control[21,22]. Sester et al[14] showed a progressive functional impairment and as well as decrease in the level of CD4 T lymphocytes in patients with renal, heart and lung transplantation[13,15].

Our study showed that low CD4 T cell count (≤200/µl) i.e. low cellular immune response was associated with significantly higher incidence of pp65 antigenemia (40%) (Table-II), while the low CD4 cell count was highly associated with high level pp65 antigen (75%) (Table III). Comparable study reports are not available in local population. Sester et al[13] showed that a loss of CMV-specific CD4 T cells in the first months after transplantation of CMV seropositive renal transplant recipients not only correlates with an uncontrolled viral replication but also with an increased incidence of CMV-related disease. In another study it was shown that a decrease in the level of CMV specific CD4 T cells correlated with impaired CMV control and long-term susceptibility to CMV Infection after kidney, heart and lung transplantation[15].

Among adults with advanced HIV-1 infection, approximately 10%-30% developed CMV disease per year[23]. Risk of CMV disease is linked closely with immune impairment as reflected by low $CD4^+$ T cell counts. A cohort study of over 1,000 adults with $CD4^+$ T counts below 250/mm^3 who were on zudovudine, observed that 109 developed CMV disease[4]. Among patients with <100 $CD4^+$ T cells/µl at enrollment, 21.4% developed CMV disease within 2 years, compared with 10.3% for patients with >100 $CD4^+$ T cells/mm^3. Francisci et al[23] in their study included only patients with a $CD4^+$ T cell count <150/µl of blood. In their study among the 65 HIV infected patients, 24 (36.92%) showed positive antigenemia and 11 (45.83%) of the 24 positive patients had high level of antigenemia. Manfredi et al[24] showed pp65 positive antigenemia were associated with low $CD4^+$ T cell count in AIDS patients. However they included patients with <100 $CD4^+$ T cells count. Among the 189 HIV infected patients 33 (17.5%) showed positive antigenemia. 20 (60.6%) of the 33 CMV infected patients with <100 $CD4^+$ T cell count showed high level of antigenemia in their series. Thus the quantitative antigenemia test can be used to select patients whose $CD4^+$ T cell count <50 or <100/µl of blood for primary prophylaxis. This could reduce both the number of treated patients and the duration of prophylaxis with ganciclovir[25].

In this study, it was observed that HIV infected patients, kidney transplanted patients and patients with malignant disorder of blood were all immunocompromised with low $CD4^+$ T lymphocyte count indicating low immunity. It was also observed that lower cellular immune response was associated with higher incidence of pp65 antigenemia as well as higher level of pp65 antigenemia, indicating more severe infection. However further extensive study with larger samples should be carried out for confirmation of these findings as well as clinical outcome of lower $CD4^+$ T cell count and higher level of CMV pp65 antigen should be delineated.

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