A Study on Seroprevalence of Cytomegalovirus among HIV-Positive Individuals

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Abstracts: Introduction: The AIDS pandemic has created an explosive increase in the number of profoundly immunocompromised people worldwide. It has been suggested that cytomegalovirus (CMV) can stimulate HIV replication either directly by trans-activating HIV genome or indirectly through the production of cytokines or up-regulation of CD4 receptors and Fc receptors. Methods: The 500 individuals who were positive for HIV infection and Three hundred healthy age and sex matched HIV-negative controls were selected and further tested for the presence of anti CMV antibodies. Results: Anti-cytomegalovirus antibodies could be detected in 68.6% HIV positive patients and in 49.33% HIV-negative controls. The difference in positivity of viral agents studied in HIV positive patients was highly significant as compared to HIV-negative individuals ($p < 0.001$). This study concludes that the seroprevalence of CMV antibodies is significantly higher among HIV- positive patients as compared to HIV negative controls, also that the increased seroprevalence of CMV antibodies is observed in persons who acquire HIV-I infection through heterosexual route (72.41%). Conclusion: The evidences to suggest that CMV can act as co-factor in HIV infection and may accelerate its progression to AIDS and facilitate its spread through sexual contact in co-infected patients, it is necessary that the co-infection should be diagnosed and treated at an earliest. This is an important step for intervention in the progression of the disease and for an overall improvement in survival of these co-infected patients.[N Sherwani, Natl J Integr Res Med, 2018; 9(2):49-54]

Key Words: Co-infection, HIV, Cytomegalovirus

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Introduction: Since its first recognition on December 1, 1981, infection with human immunodeficiency virus type 1 (HIV-1) and its end stage, acquired immunodeficiency syndrome (AIDS) still continues to be one of the major public health challenges of modern times.[1]

Being a global pandemic, AIDS has killed more than 35 million people, making it one of the most destructive pandemics in recorded history. A total of 36.7 million people were living with HIV/AIDS at the end of year 2015. An estimated 2.1 million individuals worldwide became newly infected with HIV in 2015.[2]. It is estimated that 90% of HIV infected persons live in developing countries. India, with about 20.89 lakh cases of HIV infection, has a distinction of harboring the third highest number of these patients in the world.[3]

It has been suggested that herpes simplex virus type2 (HSV-2) and cytomegalovirus (CMV) can stimulate HIV replication either directly by trans-activating HIV genome or indirectly through the production of cytokines or up-regulation of CD4 receptors and Fc receptors. Thus these viruses act as cofactors in HIV disease, which may accelerate progression to the AIDS.[4,5] Thus these viral infections should be targeted as a modifiable risk factors for HIV acquisition and progression by testing, counseling and preventing acquisition through behavioural interventions, treatment and antiviral suppression.[6]

Of the various opportunistic infections which occur in AIDS patients, cytomegalovirus disease occurs with very high frequency, as evidenced by virus isolation and autopsy findings. The frequency of occurrence of detectable CMV antibodies is also greater in AIDS patients than in HIV-negatives.[7] Several studies have also suggested that CMV co-infection may enhance HIV-1 shedding and increase the risk of progression to AIDS[8].

Halbert SP et al reported seroprevalence of 93.9% and 98% for CMV and HSV-2 respectively among HIV-positive individuals. Some studies observed the CMV seropositivity of 90.3% and 85.71% among HIV-positive individuals.[7,9,10]

The intricacies of interaction of CMV with HIV at the cellular level and the possibility of adverse outcome leading to heightened activity of either virus with disease progression are an alarming situation. Hence knowledge about the co-existence of HIV with CMV is not only essential but is mandatory to monitor the disease progression and more importantly in the treatment of these infections, where the specific therapy is identified and available.

The literature regarding the prevalence of co-infection of CMV in HIV positive individuals from India is sparse. Hence, the present study was undertaken to study the seroprevalence of CMV in HIV-positive individuals attending the Integrated Counseling and Testing Centre (ICTC) in Mayo General Hospital of Nagpur City, Maharashtra, India.

Methods: The present study was conducted in the Department of Microbiology, Indira Gandhi Government Medical College and Mayo General Hospital, Nagpur (Maharashtra).

All the patients attending ICTC were included in the study and a detail history from all the patients were taken according to a predesigned proforma. All the patients were provided with HIV pretest Counseling and the written consent was taken for testing. HIV testing was done as per NACO guidelines.

All the individuals who were positive for HIV infection were selected for this study and further tested for the presence of anti CMV antibodies. Three hundred healthy age and sex matched HIV-negative controls were also included in the study.

A total of 2250 & 300 blood samples were collected from study subjects and control respectively. All the serum samples were subjected to the detection of HIV-I and HIV –II antibodies as per NACO guidelines. [11] The serum samples found positive for HIV infection were further tested for the presence of anti CMV antibodies by using a third generation ELISA test kit ASIA–LION CMV IgG, provided by Asia–lion, Biotechnology Co. Ltd., USA.

Samples positive for anti CMV antibodies by first test were retested for confirmation of results.

The serum samples of control cases were also tested for presence of anti CMV antibodies.

Data was compiled in MS Excel and checked for its completeness and correctness. Then it was analyzed using online statistical calculator and chi square test were applied with value of < 0.05 was considered statistically significant for interpretation of finding.

Result: The HIV testing is done in 2250 patients attending ICTC, a total of 500 (22.22%) patients were positive for HIV antibodies.

Table No 1: Age & Sex wise distribution of HIV positive patients and HIV negative controls

Age (years)	HIV positive (n=500)			HIV negative (n=300)		
	Male	Female	Total (%)	Male	Female	Total (%)
< 15	28	17	45 (9.00)	16	9	15(8.33)
15 – 20	10	6	16 (3.20)	7	3	10(3.33)
21 – 30	115	53	168(33.60)	79	27	106(35.33)
31 – 40	102	92	194(38.80)	64	49	113(37.68)
> 40	60	17	77 (15.40)	37	9	46(15.33)
Total	315	185	500 (100.0)	203	97	300(100.0)

Out of 2250 patients attending ICTC, a total of 500 (22.22%) patients were positive for HIV antibodies. Age and sex matched HIV negative 300 persons (as a control) were also included in the study. Maximum numbers of HIV positive patients (38.8%) were in age group of 31 – 40 years, followed by age groups of 21 – 40 years which had 33.6% of HIV positive patients. Present study showed male preponderance in HIV positive patients. Out of total HIV positive patients, 63% were males and among HIV negative controls, 67.67% were males.(Table-1)

Among HIV positive cases, maximum number of cases, 169 (33.80%) were agricultural or unskilled workers (laborers) followed by housewives (21.40%), drivers /cleaners (10.60%) and service class people (7.0%). 87% acquired HIV through heterosexual contact and 0.4% acquired HIV through homosexual contact (MSM) among HIV positive patient. Vertical route of transmission was observed in 8% of HIV positive patients, whereas 2% of HIV positive patients acquired HIV via blood tranfusion. In 2.6% patient's route of transmission of HIV could not be identified. (Table-2)

Table No 2: Occupation-wise distribution & Probable route of transmission of HIV in HIV positive cases

Occupation	HIV positive (n=500)		
	Male	Female	Total (%)
Agriculture / unskilled worker	129	40	169 (33.80)

Truck / Auto / Taxi driver & Cleaner	53	0	53 (10.60)
Industrial /Factory worker	25	0	25 (05.00)
Hotel staff	17	0	17 (03.40)
Service class	22	13	35 (07.00)
Business	11	0	11 (02.20)
Unemployed	31	8	39 (07.80)
Student	10	9	19 (03.80)
Housewife	0	107	107 (21.40)
Others	17	8	25 (05.00)
Probable route of transmission of HIV			
Heterosexual	275	160	435 (87.0)
MSM*	2	0	2 (0.40)
Vertical	25	15	40 (8.00)
Blood transfusion	5	5	10 (2.00)
IDU**	0	0	0 (0.00)
Unknown	8	5	13 (2.60)
Total	315	185	500 (100)

* MSM - Men who have sex with men

** IDU - Intravenous drug use

Table No 3: Age-wise distribution of CMV positive cases among HIV positive patients and HIV- negative controls

Age (years)	HIV positive (n=500)		HIV negative(n=300)	
	Total	Anti-CMV positive (%)	Total	Anti-CMV positive (%)
< 15	45	20 (44.44)	25	16 (64.00)
15 – 20	16	10 (62.50)	10	6 (60.00)
21 – 30	168	115 (68.45)	106	51 (48.11)
31 - 40	194	144 (74.23)	113	52 (46.02)
> 40	77	54 (70.13)	46	23 (50.00)
Total	500	343 (68.60)	300	148 (49.33)

Among HIV positive patients, CMV antibody prevalence was highest in age group of 31 – 40 years (74.23%), followed by age group of > 40 years (70.13%).(Table-3)

Table No. 4: Gender-wise distribution of CMV positive cases among HIV-positive patients and HIV-negative controls

Gender	HIV positive(n=500)		HIV negative (n=300)	
	Total	Anti- CMV positive (%)	Total	Anti- CMV positive (%)
Male	315	218 (69.21)	203	101 (49.75)
Female	185	125 (67.56)	97	47 (48.45)
Total	500	343 (68.60)	300	148 (49.33)

Among HIV positive patients, a total of 343 (68.6%) patients had CMV antibodies. Seroprevalence of CMV antibodies was marginally higher in males (69.21%) compared to females (67.56%).(Table-4)

Table No 5: Probable route of transmission in CMV positive cases among HIV positive patients

Route	HIV positive(n=500)	
	Total	Anti-CMV positive (%)
Heterosexual	435	314 (72.41)
MSM	2	1 (50.00)
Vertical	40	16 (40.00)
Blood transfusion	10	2 (20.00)
IDU	0	0 (00.00)
Unknown	13	9 (69.23)
Total	500	343 (68.60)

Out of 435 HIV positive heterosexuals, 314 (72.41%) patients were having anti-CMV antibodies. Fifty percent of HIV positive MSM were positive for anti-CMV antibodies.

Among vertically acquired HIV positive patients, 40% had anti-CMV antibodies. Out of 13 HIV positive patients with unknown route of transmission 9 (69.23%) had CMV antibodies. Twenty percent of transfusion associated HIV positive patients had CMV antibodies. (Table-5)

Table No 6: Seropositivity of CMV in HIV positive patients and HIV negative controls

Viral agent	HIV positive n=500 (%)	HIV negative n=300 (%)	Chi square test, d.f., p value
Anti CMV antibodies	343 (68.6)	148 (49.33)	X2=29.360 d.f.=1 p < 0.001

Anti-CMV antibodies were present in 68.6% HIV-positive patients and in 148 49.33% HIV-negative controls. (Table-6)

The difference in positivity of the viral agent studied in HIV positive patients was highly significant as compared to HIV-negative individuals.

Discussion: The AIDS pandemic has created an explosive increase in the number of profoundly immunocompromised people worldwide. In India HIV infection is one of the major infectious diseases. Being chronic life-long in nature; its impact is huge compared to other infectious disease.

The profound immunodeficiency caused by infection with HIV results in defects in cellular immunity to common infectious agents. CMV is found to reactivate more frequently in the context of severe HIV- induced immunosuppression.[12]

Primary infection with CMV in HIV positive patients in unusual; it usually remains latent in body. With increasing impairment in cell mediated immunity (CMI), reactivations occur resulting in clinical syndromes that include retinitis, oesophagitis gastritis, polyradiculitis, hepatitis and adrenalitis. HIV positive patients in whom CD4 cells are decreased fewer than 100 cells/ cu. mm have a significantly increased risk for the development of serious CMV disease.[13,14]

It has been suggested that like HSV-2, CMV also acts as a co-factor in HIV disease, and may accelerate the progression to the AIDS[5]. Several groups have reported interaction at a molecular level between HIV and CMV, suggesting that CMV transactivator gene, in addition of up-regulating the CMV genome, can also up-regulate HIV replication in HIV–CMV co-infected patients.[4]

CMV infection is associated with increased production of several cytokines including TNF-α, IL-6, and IL-8. IL-8 has been reported to enhance HIV-1 replication. Thus up–regulation of cytokines is a potential mechanism for CMV interaction with HIV-1 in co-infected compartments.[8] Thus CMV co-infection may enhance HIV shedding and increase the progression to AIDS.[9,15]

Of the various opportunistic infections which occur in AIDS patients, CMV disease occurs with very high frequency as evidenced by virus isolation and autopsy findings. The frequency of occurrence of detectable CMV antibodies is also greater in AIDS patients than in HIV-negative individuals.[7] In the present study the HIV- CMV co-infection (by virtue of anti-CMV antibody positivity in HIV-infected patients) was observed among 68.6% of HIV-positive patients. This was significantly ($P<0.001$) higher than the CMV positivity (49.33%) among HIV-negative control cases (Table -6). Stanekova D et al (2006), Clarke LM et al (1996), and Halbert SP et al (1986), observed seroprevalence of CMV antibodies in 85.71%, 90.30%, and 93.9% among HIV- positive individuals.[7,9,10]

Fabio G et al (1997) and Broccolo F et al (2002) have reported CMV–DNA by PCR in 17% and 48% of HIV positive individuals. In both the studies CMV detection was associated with low CD4 counts. Although the PCR assays do not directly detect active infection, the DNAemia is likely to represent an increase viral burden in blood as a result of virus replication. In both the above quoted studies the presence of CMV-DNA in plasma was significantly associated with CMV-disease.[12, 13]

Our study observed a slightly higher seroprevalence of CMV antibodies among males HIV patients (69.21%) than in females (67.56%) (Table-4).

In the present study maximum number (74.23%) of HIV patients with positive CMV antibodies belonged to age group of 31-40 years (Table-3). Seroprevalence of HIV antibodies in present study, was found to be highest (72.41%) in heterosexually acquired HIV-positive individuals compared to HIV positive patients acquiring disease through other modes of transmission (Table-5).

As droplet infection (close contact) is the major route of transmission of CMV infection[16], it is difficult to rule out this mode of transmission of CMV among HIV-positive individuals. This view is further supported by the findings that the 69.23% of HIV positive patients with unknown mode of acquiring HIV were also positive for CMV antibodies (Table5).

High rates of CMV co- infection in HIV positive patients with sexual promiscuity is after all a matter of worry, as there are studies suggesting that CMV from infection enhances HIV shedding from genital mucosal surfaces, thus increasing the chances of effective transmission to the sexual partners.[9]

Infection with CMV is ubiquitous in most HIV- infected populations. Seropositivity among healthy homosexual men exceeds 90%, and among homosexual men with HIV infection, seropositivity approaches 100%. Excretion of CMV is common, especially in semen, which may account for high rate of transmission among homosexual men.[17] In present study, one (50%) out two of the HIV positive men who have sex with men (MSM) was positive for CMV antibodies. Levy E et al (2005) and Gallant JE et al (1992) both reported CMV seroprevalence of 100% among HIV positive homosexual men.[17, 18]

Thus CMV has several possible roles in the pathogenesis of AIDS. CMV causes a number of clinical syndromes in patients infected with HIV. In addition, CMV may potentiate the cellular immunodeficiency observed in patients with HIV infection either directly or through enhancement of HIV replication. Finally CMV may predispose the host to bacterial or fungal infection by compromising the integrity of mucosal barriers to infection.[19,20.21] Thus identification of HIV-CMV co-infection may help in understanding the pathogenesis of AIDS, and may provide an important opportunity for intervention in the progression of the disease at an early date.

Conclusion: A significantly higher seroprevalence of CMV antibodies among HIV infected patients is found in our region indicating a frequent possibility of reactivation of CMV in context of immunosuppression due to AIDS. As there are evidences to suggest that CMV can act as co-factor in HIV infection and may accelerate its progression to AIDS and facilitate its spread through sexual contact in co-infected patients, it is necessary that the co-infection should be diagnosed and treated at an earliest, which is an important step for intervention in the progression of the disease and for an overall improvement in survival of these co-infected patients. Thus the study suggests more careful and a vigilant screening of CMV in HIV-infected patients. The finding of the present study will be useful for public health authorities in priority settings and resource allocation under National AIDS control programme in Nagpur city, Maharashtra.

Acknowledgment: The authors are highly thankful to the faculties of department of microbiology, Indira Gandhi government medical college and staff of ICTC centre, Mayo hospital, Nagpur (Maharashtra), India for their cooperation and support during the entire study period. Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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Conflict of interest: None
Funding: None
Cite this Article as: N Sherwani, R Khadse, S Jalgaonkar. A Study on Seroprevalence of Cytomegalovirus among HIV-Positive Individuals. Natl J Integr Res Med 2018; 9(2):49-54