Original Article

Microbiology

Correlation of Serology, Tissue Culture and PCR in Identification of Herpes Simplex Type-2 infection among HIV Patients

SHAMEEM BANU A.S, LAKSHMI S., KAVERI K., JAYAKUMAR S.

ABSTRACT

Background: Herpes infection in Acquired Immunodeficiency Syndrome (AIDS) patients is more severe and in atypical location. Diagnosis becomes difficult as the clinical presentation tend to be atypical. There are numerous laboratory methods to identify Herpes Simplex Virus (HSV) infection and it also becomes essential as a early initiation of antiherpetic treatment has shown to decrease Human Immunodeficiency Virus (HIV) replication and acyclovir resistance. In this study we have correlated serology of HSV type 2 with tissue culture and Polymerase Chain reaction (PCR).

Materials and Methods: This study was performed in the Institute of Microbiology, Madras Medical College and the Department of Virology, King Institute of Preventive Medicine. The sample size was 60 cases all with genital lesions such as ulcers and vesicles were selected. Out of which 30 were known HIV positive and 30 were HIV negative attending out patient Sexually Transmitted Diseases Department, Government General Hospital (GH) Chennai. Thirty cases of blood donors were taken as control population from the Blood Bank, GH Chennai. Laboratory diagnostic procedures such as serological tests, virus culture, immunoflourescence and PCR were done keeping PCR as a gold standard test for diagnosing HSV infection.

Results: Out of 60 patients, 30 were HIV positive and remaining negative. Among HIV positive patients 56.6% had IgM antibody and 83.3% had IgG antibody, were as among HIV negative patients IgM were 36.6% and 70% had IgG. Control group serological parameters for IgM, IgG HSV-2 were 6.6% and 56.6% respectively. Virus isolation was positive for three samples and PCR for six samples. On comparing with gold standard test, sensitivity and specificity for serology is 100% and 59%, for tissue culture the sensitivity is 50% and specificity is 100%.

Conclusion: HSV 2 antibody detection by type specific serology kit can be used as an effective tool in screening infection both in typical and atypical presentations before initiating treatment, as antibody detection is much easier and feasible screening test when compared to PCR and tissue culture.

Key Words: Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), Tissue Culture, Polymerase Chain Reaction

KEY MESSAGE

- Clinical presentations of Herpes Simplex Virus (HSV) infection tend to be atypical in HIV patients.
- Laboratory screening test or diagnostic test for Herpes Simplex Virus (HSV) infection is essential before initiating antiherpetic treatment.

INTRODUCTION

Since AIDS was first recognised 25 yrs ago, remarkable progress has been made in improving the quality and the duration of life for HIV infected persons. Improved recognition of opportunistic infection disease processes, improved therapy for acute and chronic complications and introduction of chemoprophylaxis against key opportunistic pathogens. The sexual transmission of the Human Immunodeficiency Virus (HIV) is facilitated by the presence of genital ulcer disease. Herpes Simplex Virus type 2 (HSV-2) is the most common cause of genital ulcer disease in both the developed and the developing countries. HSV-2 and its potential interaction with HIV have emerged as a major public health problem for countries which are facing the global HIV -1 pandemic [1].

The transmission of HIV-1 to sexual partners may also be aided by the presence of genital ulcers. High levels of HIV-1 have been documented in the HSV lesions. Furthermore, HSV reactivates more frequently in persons with the HIV-1 infection. These findings indicate a higher risk of HIV-1 exposure for individuals whose sexual partners have both the HIV-1 and the genital herpes infections [2].

Up to 90% of the people with the HIV infection also have the HSV-2 infection. Most of the people who are infected with HSV-2 do not know that they have the virus because its symptoms can be mild or they may be absent. The HSV-2 infection can cause recurrent sores and breaks in the skin of the genital region, which can be mild and can often go unnoticed. The HSV-2 infection also attracts immune cells which are called the CD4 T-cells to the genital region, which HIV uses to establish or pass the infection. Multiple studies have shown that frequent genital herpes recurrences increase the amount of HIV in the blood and the genital tract. The HIV virus is also shed from genital herpes ulcers and persons with such ulcers can transmit HIV to others more efficiently [3].

The herpes infections are more severe and atypical in AIDS patients. Multiple necrotic ulcers may be present in atypical locations. The

outbreaks may progress to more extensive and persistent locations. There may be more frequent recurrences or chronic and nearly continuous ulcerations as the HIV related immunosuppresion progresses. Patients with the human immunodeficiency virus (HIV) infection who experience the first or recurrent HSV-2 episodes can develop severe and extensive lesions, which may become difficult to control by the standard antiviral therapy [4]. In some cases, a visceral spread can also occur [2].

The detection and the treatment of sexually transmitted diseases such as genital herpes actually decrease the rate of the HIV infection. The treatment of the HSV infection in HIV seropositive patients suppresses the bursts of the HIV replication which occur during the active herpes infection. All the five trials demonstrated significant reductions in both the genital and plasma HIV replication after one to three months of daily treatment with either valacyclovir or acyclovir [5]. As the presentation tends to be atypical, a diagnosis which is based on clinical grounds becomes difficult and so laboratory diagnostic methods become essential, as initiating the antiherpetic treatment has been shown to decrease both the HIV replication and the emergence of acyclovir resistance in these patients.

Various tests are available for diagnosing HSV-2 infections in the laboratory, with many pros and cons. In this present study, we correlated PCR, tissue culture and serology tests to find out an ideal and feasible tool for diagnosing the HSV type 2 infection.

PATIENTS, MATERIALS AND METHODS

Study Participants and Design

This study was performed at the HIV Reference Centre, Institute of Microbiology, Madras Medical College, Chennai and in the Department of Virology, King Institute of Preventive Medicine, Guindy. The study group was the patients who attended the Out Patients Sexually Transmitted Diseases (STD) Department, Government General Hospital, Chennai and the control population was obtained from the Blood Bank, Government General Hospital, Chennai.

The study population represented both the male and female patients who attended the STD clinic. The patients were interviewed regarding their demographic characteristics, their medical history, their HIV/AIDS status knowledge, and their clinical symptoms. In brief, all the patients received pre-and post-test counseling for HIV and those who gave consent for the study were included.All the participants were informed about the confidentiality of their test results. PCR was the gold standard test, with which we compared tissue culture and serology.

Specimen Collection and Laboratory Tests

The sample size was 60 cases (with vesicles or ulcer) and 30 controls. Blood specimens which were obtained by venipuncture were tested for HIV, IgM and IgG for HSV-2. Sterile cotton swabs were used to collect scrapings from the base of the vesicular and the ulcerative lesions for the virus isolation and the PCR assays.

Serological testing for HIV: The serum samples were screened by using a commercially available EIA kit for the identification of the HIV-1 and the HIV-2 antibodies (LAB Systems HIV-EIA, an indirect, solid phase immunoassay).

Serologic testing for HSV-2:[6] The serological testing for HSV-2 was performed on all the specimens by using IgM type specific ELISA (NovumHSV2 IgM immunoassay) and Herpes simplex virus type 2 IgG ELISA (NovumHSV2 IgG immunoassay). Positive and negative control serum samples were used in each experiment. The cut-off was determined by dividing the optical density (OD) of the positive and the negative controls. The average absorbance (OD) value of the cut-off serum which was run in duplicate was calculated. The cut off index (COI) of each serum sample was determined by dividing the OD which was obtained for that serum sample, by the average OD of the cut-off serum. A COI below or equal to 1 was considered to be negative, that above 1.1 was considered to be positive and a COI between 1 and 1.1 was considered as borderline.

Preparation of the Specimen for the Virus Isolation and for PCR Amplification

A swab was immediately transferred into 2ml of viral transport medium and transported to the laboratory in a cold chain. The specimen was processed by using a standard protocol and it was inoculated immediately for culture. The remaining was stored at -70 deg C for PCR testing later.

Virus Isolation [7]

0.2 ml of each of the processed samples was inoculated into tubes which contained a confluent verocell monolayer (Vervet monkey kidney cell lines in Minimal Essential Medium which was supplemented with 2% foetal calf serum) for virus isolation by using standard tissue culture techniques. The cultures were investigated daily by using an inverted microscope to see the cytopathological effect (CPE) for a week.The cytopathic effects which were produced by the Herpes Simplex virus were ballooning of the infected cells and the formation of multinucleated giant cells. The tubes which showed the cytopathic effect were confirmed by immunoflourescence. Staining was done with fluorescent iso thiocanate which was conjugated to HSV2 polyclonal antiserum (reagent obtained from J. Mitra). Prototype Herpes Simplex 2 strains were used as positive controls and uninfected cell cultures were used as negative controls. Specific fluorescence was detected.

PCR Amplification [8]

The stored, processed samples were centrifuged and 50µl of the supernatant was added to an equal volume of DNA extraction buffer. The DNA extraction was done by suing a NeoDin HSV Screen and Type I, II PCR Kit-One tube nested PCR kit. The target region was the nucleotides and the nucleosides of the gD gene of Herpes Simplex from the clinical samples. The PCR amplified product was electrophoresed and the product size was analyzed by using a UV transilluminator. The product size of the sample was compared with the positive and negative controls which were provided in the kit and also with the molecular weight markers.

RESULTS

Ninety subjects were enrolled in the study, of which 30 were HIV positive (20 males and 10 females) and 30 were HIV negative individuals (19 males and 11 females) who attended the STD clinic and 30 cases (14 males and 16 females) attended the Blood Bank, which were taken as the control group.

In the HIV positive study group, the incidence of the genital lesions was 50% in the 20-29 years age group and it was 50% in the 30-39 years age group. Out of the 60 patients, 50 patients presented with ulcers (22 - HIV positive and 28 - negative) and 10 patients with vesicles (8- HIV positive and 2- HIV negative).

The seroprevalence of the HSV-2 antibody (IgM, IgG) among the HIV positive and negative patients as compared to the control group is shown in [Table/Fig-1].

The virus was isolated only in 3 cases (5%), for which the cytopathic effect was confirmed by direct immunoflourescence.

A total of 6 cases (10%) were positive for PCR. The positivity results for the virus isolation and for PCR among the HIV cases with vesicles and ulcers with respect to the treatment is shown in [Table/Fig-2].

The sensitivity and the specificity for serology and tissue culture were computed by using the 2 by 2 table as shown in [Table/Fig-3]. The statistical analysis for the sensitivity and specificity of these two tests was done by using the Receiver Operating Characteristic (ROC) curve. The value for the serology of IgM by using the ROC curve was 0.796 and for tissue culture, it was 0.750. The ROC curve is shown in [Table/Fig-4].

Serology test	HIV Positive	HIV Negative	Control
IgM HSV-2	17 (56.6%)	11 (36.6%)	2 (6.6%)
IgG HSV-2	25 (83.3%)	21 (70%)	17 (56.6%)
HZV IgG	23 (76.6%)	18 (60%)	3 (10%)

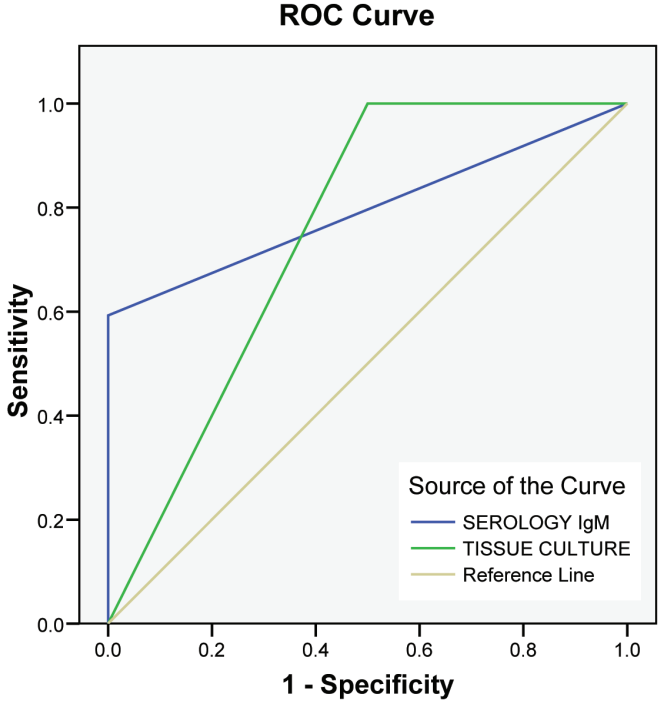
[Table/Fig-1]: Seroprevalence of HSV-2 antibody and HZV IgG- Total 90 cases

Test	HIV Positive		HIV Negative	
	Vesicles (8)	Ulcers (22)	Vesicles (2)	Ulcers(28)
Virus Isolation	2*	0	1*	0
PCR	2	2	2	0

[Table/Fig-2]: Rate of Positivity in virus isolation and PCR among HIV cases with vesicles and ulcer.
*Antiherpetic treatment not initiated.

Test	Sensitivity	Specficity
Serology	100%	59%
Tissue Culture	50%	100%

[Table/Fig-3]: Sensitivity and specificity for serology and tissue culture test in detecting HSV 2 Infection keeping PCR as gold standard



[Table/Fig-4]: Receiver Operating Characteristic (ROC) curve for serology IgM and Tissue Culture of HSV 2 Infection.

DISCUSSION

The HIV-1 epidemic in India is now 24 years old and it has spread rapidly across the country; as a result, India has the potential to have more HIV-1–infected individuals than any country in the world [1]. Among all the causes, HSV-2 is the predominant cause of genital herpes, accounting for approximately 85% of the cases of primary genital herpes and >95% of the cases of recurrent genital herpes in Sydney, Australia, and elsewhere [9].

The importance of the association between genital herpes and the HIV-I infection is underscored by the fact that the prevalence of genital herpes is much greater than the prevalence of syphilis or chancroid. Therefore, the risk of acquiring HIV-1 was increased to 2-fold in the patients with genital herpes [10].

In addition, most of the patients with genital herpes are likely to have recurrent episodes of genital ulceration. Thus, if genital herpes infections are the risk factors for the acquisition of HIV-1, they have the potential to contribute to the continued spread of the HIV-1 infection because of their recurrent nature, due to the high prevalence of genital herpes in populations who are at a risk for acquiring the HIV-1 infection and due to the large numbers of herpes-infected individuals who continue to engage in sexual activities despite their infections.

There are several possible biological mechanisms that HSV-2 could use to act as a cofactor in HIV acquisition or transmission. First, the HSV-2 reactivations result in mucosal or epithelial disruption, creating a portal of exit or entry for HIV, to which the activated CD4 cells are recruited. There also appear to be several cellular interactions that promote the establishment of the HIV infection; coinfection with HSV-2 may lead to the creation of “pseudotypes” (i.e., particles containing the HIV viral genome which are enveloped in the HSV surface glycoprotein), thus allowing HSV to infect the cells that could not be infected by HIV alone [11]. The HSV-2 infection may also promote the increased expression of the HIV target cells (i.e., the CCR5+ CD4 cells and the immature dendritic cells) [12].

The episode of acute HSV infection increases HIV transcription, as was evidenced by the intracellular HIV gag messenger RNA. Co-infection of the human CD4 cells with HSV and HIVresults in a undirectional, accelerated replication of HIV. The prevalence of HSV-2 shedding is greater in HIV seropositive individuals and it increases significantly with a decrease in the CD4 count.

There are also several mechanisms that may explain how HSV-2 increases the levels of genital and plasma HIV, thus enhancing the HIV transmission. In coinfected individuals, the HSV-2 proteins may increase the replication of HIV at the mucosal sites by trans-activation of the HIV long terminal repeat [13]. Cytokine release may also stimulate HIV replication. A recent study demonstrated that coinfection with HIV could lead to a depletion of the immune cells which are responsible for controlling the HSV-2 reactivation, resulting in an impaired immune control of HSV-2, and leading to further HSV-2 reactivation and subsequent increases in the HIV levels in the genital tract [12].

Some data suggest that the lesions which are caused by HSV may be atypical in morphology as compared to those which have been clinically described, thereby delaying or preventing an accurate diagnosis [8]. The atypical manifestations include vulvar, penile, or perianal fissures, localized erythema, and back pain without genital lesions. Both of these groups are at risk of transmitting the virus. The HSV-2 virus is capable of causing either genital or

oropharyngeal infection and can produce lesions that are clinically indistinguishable from lesions which are produced by the HSV 1 virus. Extra genital lesions are more common in women than in men. The areas which are generally involved are the buttock, groin, thigh and the fingers. The causes of the extra genital lesions are the autoinoculation of the virus, viral reactivation in another part of the dermatome and viraemic spread [2].

In this present study, the seropositivity for HSV 2 was more in the HIV positive group as compared to that in the HIV negative group (56.6% and 36.6%). This was similar to the results which were obtained in a study which was conducted at the John Hopkins University School of Medicine, Baltimore (73% and 48.9) [14].

The 28 patients who had presented primarily with vesicular and ulcerative lesions had IgM antibodies and 14 of them had IgG antibodies also. But the patients with recurrent vesicles and ulcerative lesions had IgG antibodies without the IgM antibodies.

In the control population, the IgM positivity percentage was 6.6%. The seropositivity in the control group was well supported by numerous studies which had revealed that a majority of the HSV-2 infections were left undiagnosed. In the large NHANES survey, only 9% of the persons with the HSV-2 antibodies had knowledge about their disease. Conversely, 22% of those who denied a history of genital herpes were found to be HSV-2 seropositive [2].

It has been estimated that about 20% of the patients with HSV-2 antibodies are truly asymptomatic or that they have lesions only in locations such as the cervix, that are impossible to observe [15]. The remaining 60% of the undiagnosed persons with genital HSV-2 infection have symptoms that are not recognized by either the physicians or the patients as being caused by herpes [2]. The sensitivity of antibody detection of our ELISA kit was 100%, which well correlated with the findings of a study which was done in Africa [16], where they compared various ELISA kits and found that their sensitivities ranged between 83.3 and 100%. The differences in the specificity were more marked, with estimates of between 46.6 and 97.7%. The detection of antibodies allows the diagnosis of an infection when virological methods such as culture, antigen detection, or PCR are impractical [17].

The observation which was made from this study was that, PCR (10%) was able to pick up more cases than virus isolation (5%), which was supported by Safrin et al [8] studies wherein the positivity for PCR was (50%) and that for virus isolation was (39.8%).

Moreover, in the present study, the virus was isolated (5%) from the samples which were taken from the patients with vesicular lesions, who had not initialized the antiherpetic treatment. Virus isolation was not present in the remaining cases and this was probably due to the decrease in the viral shedding after the treatment. There was no virus isolation from the ulcerative lesions with and without treatment, and this can be explained by the fact that the virus shedding from the ulcers was not consistent, whereas PCR was able to pick up cases from both the vesicles and the ulcers (10%) and even from cases where the antiherpetic treatment had been initiated. Our finding of the greater sensitivity of PCR than virus isolation for the older or crusted lesions, mirrors that of Cone et al, who showed that the mean duration of the HSV DNA positivity was more than two fold longer than of that of the virus culture [18]. Although viral culture has been widely used as a means to confirm the clinical diagnosis of the HSV infections, it has been shown to lack sensitivity when the lesion is partially healed or crusted. Improper handling of the specimen could further decrease the yield of the virus culture [17].

The selection of an effective diagnostic technique depends on a number of parameters like the clinical stages of presentation and the number of days after the clinical illness, whether it was a primary or recurrent case and whether any antiherpetic treatment had been taken or not. If a case presented within the initial 14 days with vesicles and without taking any antiviral therapy, then virus isolation could be done. On the contrary, if a case presented with ulcers and if antivirals had already been initiated for it, then PCR would be ideal.

HSV 2 antibody detection by using a type specific serology kit can be used as an effective tool in screening the infection, both in typical and atypical presentations before initiating the treatment, because it is a much easier and feasible screening test as compared to PCR and tissue culture. The most significant potential application of serology is to detect the silent carriers of HSV-2, especially in high-risk settings such as STD clinics [2]. The PCR and tissue culture techniques need a good infrastructure and the availability of trained personnel.

In cases with a high clinical suspicion, with the atypical presentation of the HSV-2 infection, IgM antibody detection can be done to initiate the empirical antiviral treatment which can help in preventing the spread of the virus. Moreover, various diagnostic methods alone cannot halt the spread of genital herpes; it is an important step in the clinical management and counseling of the patients who wish to have full information about the status of their sexual health.

REFERENCES

[1] Reynolds S J, Risbud A R, Shepherd M E, Zenilman J M, Brookmeyer R S, Paranjape R S et al. Recent Herpes Simplex Virus type 2 infection and the risk of the Human Immunodeficiency Virus type 1 acquisition in India. *J Infect Dis* 2003; 187:1513–21.

[2] Ashley R L, Wald A., Genital Herpes: Review of the epidemic and potential use of type-specific serology. *Clin Microbiol Rev* 1999; 12(1):1–8.

[3] Celum C, Wald A, Lingappa JR, Magaret AS, Wang RS, Mugo N et al . Acyclovir and the transmission of HIV-1 from persons who are infected with HIV-1 and HSV-2. *N Engl J Med* 2010; 362:427-39.

[4] Johnson, LS, Polsky B, Herpes simplex virus infection in cancer patients. *Infect Med* 1993; 10:18–52.

[5] Delany-Moretlwea S, Celum C. HSV-2 treatment interventions to control HIV: hope for the future? ftguonline.org/ftgu-232/index.php/ftgu/article/view/2000/3996.

[6] Rose NR, Friedman H, Fahey JL. Manual of Clinical Laboratory Immunology, 3rd ed. Washington, D.C.: American Society for Microbiology Press; 1986; 99-101.

[7] Freshney RI. Culture of Animal Cells: A Manual of Basic Techniques, 2nd ed. New York: Alan R. Liss Inc; 1987; 397.

[8] Safrin S, Shaw H, Bolan G, Cuan J, Chiang C S. Comparison of virus culture and the polymerase chain reaction for the diagnosis of mucocutaneous herpes simplex virus infection. *Sex Transm Dis* 1997; 24(3):176-80.

[9] Corey L. Adams HG, Brown Z A, Holmes KK. Genital herpes simplex virus infections: clinical manifestations, course and complications. *Ann. Intern. Med*. 198; 98:958-72.

[10] Morse SA, Trees DL, Htun Y, Radebe F, Orle KA, Dangor Y, et al. Comparison of the clinical diagnosis and the standard laboratory and molecular methods for the diagnosis of genital ulcer disease in Lesotho: association with the Human Immunodeficiency Virus infection. *J Infect Dis* 1997; 175:583-9.

[11] Heng MC, Heng SY, Allen SG. Co-infection and synergy of the human immunodeficiency virus-1 and herpes simplex virus-1. *Lancet*. 1994; 343(8892):255-8.

[12] Rebbapragada A, Wachihi C, Pettengell C, et al. Negative mucosal synergy between herpes simplex type 2 and HIV in the female genital tract. *AIDS*. 2007; 21(5):589-98.

[13] Mosca JD, Bednarik DP, Raj NB. Herpes Simplex Virus type-1 can

reactivate the transcription of the latent human immunodeficiency virus. *Nature.* 1987; 325(6099):67-70.
[14] Hook EW, Cannon RO, Nahmias AJ, Lee FF, Campbell CH, Jr., Glasser D, et al. Herpes Simplex Virus infection as a risk factor for the Human Immunodeficiency Virus infection in heterosexuals. *J Infect Dis* 1992; 165: 251-5.
[15] Corey, L. The current trend in genital herpes. Progress in prevention. Sex. *Transm Dis* 1994; 21(2):38–44.
[16] Dyck EV, Buve A, Weiss HA, Glynn JR, Brown DWG, Deken B D,Parry J, Hayes RJ, et al. Performance of commercially available enzyme immunoassays for the detection of antibodies against Herpes Simplex Virus Type 2 in African populations. *J Clin Microbiol* 2004; 42(7): 2961-65.
[17] Koutsky LA, Steven CE, Holmes KK. The underdiagnosis of genital herpes by the current clinical and viral-isolation procedures. *N Engl J Med* 1992; 326:1533-9.
[18] Cone RW, Hobson A C, Palmer J, Remington M, Corey L. Extended duration of Herpes Simplex Virus DNA in genital lesions which were detected by the polymerase chain reaction. *J Infect Dis* 1991;164(4): 757-60.

AUTHOR(S):

1. Dr. Shameem Banu A .S
2. Dr. Lakshmi S.
3. Dr. Kaveri K.
4. Dr. Jayakumar S.

PARTICULARS OF CONTRIBUTORS:

1. MBBS, MD (Microbiology),
   Department of Microbiology,
   Saveetha Medical College & Hospital, Saveetha University,
   Thandalam, Kancheepuram District – 602 105
   Tamil Nadu, India.
2. MBBS, MD (Microbiology),
   Department of Microbiology,
   Trichy Medical college,Trichy,
   Tamil Nadu, India
3. MBBS, MD (Microbiology),
   Deputy Director, Virology Section, Department of Preventive Medicine, King Institute, Guindy,
   Tamil Nadu, India
4. MBBS, MD (Microbiology),
   Department of Microbiology,
   Saveetha Medical College & Hospital, Saveetha University,
   Thandalam, Kancheepuram District – 602 105
   Tamil Nadu, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Shameem Banu A .S.
Professor & Head, Department of Microbiology,
Saveetha Medical College & Hospital, Saveetha University,
Thandalam, Kancheepuram District – 602 105
Tamil Nadu.
Phone: 9940127670
E-mail: shameembanu10@rediffmail.com

DECLARATION ON COMPETING INTERESTS:

No competing Interests.

Date of Submission: **Jun04, 2011**
Date of peer review: **Jul19, 2011**
Date of acceptance: **Aug19, 2011**
Date of Publishing: **Nov 11, 2011**