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Research Paper

SEROPREVALENCE OF HIV IN PATIENTS ATTENDING VCTC IN A TERTIARY CARE HOSPITAL AND SPECTRUM OF OPPORTUNISTIC INFECTIONS AND PROFILE OF CD4 COUNTS AMONG HIV PATIENTS AND MOLECULAR CHARACTERISATION OF HIV

MEDICAL SCIENCE

KEYWORDS : HIV, Seroprevalence, CD4 Count, Opportunistic Infections.

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ABSTRACT

This study was conducted to evaluate the seroprevalence of HIV infection in Thanjavur by subjecting the serum samples to Rapid Card tests, confirm by ELISA and determine the CD4counts for reactive patients. To categorise the cases according to the presenting complaints and screen for Opportunistic Infections and do Molecular Characterisation for HIV-1. 11953 patients were screened for HIV by COMB-AIDS and reactive samples subjected to HIV Triline, HIV Trispot & ELISA. In which 346 were reactive andCD4 count estimated. Zeihl –Neelsen staining of sputum, culture of oral swabs for Candida, Toxoplasma, HSV-2screening by ELISA, Cryptococcal latex agglutination test for reactive cases and Molecular Characterisation of HIV-1 for 10 samples were done. Seroprevalence of HIV was 2.8%. Oral Candidiasis (39.02%) emerged as the most common Opportunistic Infection followed by Pulmonary Tuberculosis (28.03%), HSV -2 (14.45%) Toxoplasmosis (5.78%) and Cryptococcosis (3.41%). All 10 samples answered positive in PCR.

INTRODUCTION

Infection with Human Immuno Deficiency Virus and its end stage Acquired Immuno Deficiency Syndrome are the major public health challenges of modern times with 25 million people already dead and 30 to 40 million people living with HIV/AIDS(9). The illness was first described in 1981 and HIV-1 was isolated at the end of 1983. Since then AIDS has become a pandemic. AIDS is one of the most important public health problems worldwide at the start of 21st century (6).

The first case of AIDS in India was reported in 1986 and now India ranks second among World countries in HIV infection. India has an estimated 2.3 million HIV positive persons. Sexual route appears to be the major mode of transmission though injectable drug use is also emerging as an important mode of transmission in some parts of the country (7). Overall the average prevalence rate of HIV in India is 0.9% and it accounts for 10% of global HIV burden (10).

HIV infects the cells and starts to replicate in the CD4 T cells and macrophages. It induces the body's immune system to produce antibodies specific to HIV. The period between acquisition of infection and production of detectable HIV antibodies is called window period lasting for 2 to 12 weeks. During this period the person is highly infectious but may not test positive for common HIV antibody tests (10).Because of the progressive destruction of CD4 lymphocytes and other immune cells, there is decline of immune response patients with HIV are extremely susceptible to a variety of opportunistic infections (7).

In India TB is the most common opportunistic infection among the HIV infected individuals. Other commonly reported opportunistic infections include Oral Candidiasis, Herpes zoster, Cryptococcus meningitis, Cerebral toxoplasmosis and Cytomegalovirus retinitis. The introduction of ART has dramatically reduced the incidence of opportunistic

infections among the HIV infected individuals(5,11).The initiation of primary prophylaxis for opportunistic infections is based chiefly on CD4 count which has shown to be an excellent predictor of short term overall risk of developing AIDS among HIV infected patients(10).

The lab diagnosis of HIV infection is based on the detection of HIV antibodies. A variety of HIV antibody assays are available like ELISA, Western blot and Rapid tests.HIV infection cannot be successfully diagnosed during the window period using antibody based assays. The tests employed are p24antigen and HIV proviral DNA assays. The proviral DNA assays is based on PCR which is highly sensitive and specific (10).

Hence this study was undertaken to screen the patients for HIV infection and assess the clinical profile with respect to the presenting symptoms, CD4 count so that Opportunistic infections can be diagnosed early and treated thereby quality and expectancy of life can be improved.

MATERIALS & METHODS

This study was conducted among the cases attending Voluntary Counseling Testing Centre, Thanjavur Medical College Hospital. The study period was 1 year from September 2012 to August 2013.The study population includes 346 HIV positive patients detected by antibody test. It is an observational study. Written consent to participate in the study was obtained from the patients. This study was reviewed and approved by our Institutional Ethical Committee. All data were handled confidentially and anonymously. After giving pretest counseling 5ml of venous blood was collected from the patients with universal precautions and transferred to sterile screw capped vials. The blood samples were transported to the lab immediately in an ice box with proper labeling and packing. The blood samples were allowed to clot by placing in a rack at room temperature (20 - 25°C) f or at least 30 minutes. After that serum was separated by centrifugation. The clarified serum was then transferred to a sterile vial and was stored at +4°C (short term storage) and at -70°C (long term storage). Sputum samples were collected from patients with symptoms of cough, fever and weight loss. Oral swabs were taken from those with oral thrush.

All serum samples were screened by COMB-AIDS kit . Those samples which test reactive to COMB-AIDS kit were subjected to PAREEKSHAKHIV 1/2 TRILINE CARD test .Those samples which test reactive to the second test were subjected to AIDSCAN HIV 1/2TRISPOT TEST KIT procedure. The samples were further confirmed by HIV ELISA. CD4 counts of the reactive patients were detected by BD-FACS counter. The sputum samples were screened for Pulmonary Tuberculosis by Zeihl – Neelsen staining. Oral swabs were streaked on SDA. All the reactive samples were screened for Toxoplasmosis by Toxoplasma IgG ELISA, HSV-2 by HSV-2 IgG ELISA and Cryptococcosis by Latex Agglutination Test. AMPLICOR HIV-1 DNA PCR, version 1.5 was run for 10 samples to detect HIV-1 DNA.

RESULTS

This study was carried out among 11953 cases and analysed over a period of one year from September 2012 to August 2013. Of the 11953 cases, 346 cases were found to be reactive for HIV by Comb AIDS, HIVTriline , HIV Trispot and confirmed by HIV Microlisa. In 346 reactive cases 345 cases were reactive for HIV-1, 1 case was reactive for both HIV-1 & HIV-2. The prevalence of HIV in this study over a period of one year was 2.8%. Out of the 346 reactive cases 207(59.83%) were males and 139

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(40.17%)were females. The male to female ratio was 1.48:1.The majority of the reactive cases, 146 (42.20%) out of 346 were in the age group of 31to 40 years followed by 84 cases(24.28%) in the age group of 41 to 50 years. Among the reactive 8 (2.31%) were children. **Table: 1**

On the basis of habitat, people coming from rural areas (77.17%) outnumbered the urban population (22.83%).The education wise distribution showed that 113(32.66%) were illiterates 123(35.55%) were educated up to primary level, 68(19.65%) were educated up to secondary level and35(10.12%) were graduates. Among the males, majority were farmers(35.27%) followed by laborers(33.33%) and drivers(17.40%). Among the females, majority were house wives(88.49%) . The distribution of the subjects showed that 312(90.17%) out of 346 were married and 21(6.07%) were unmarried. Heterosexual route (97n.11%) was the major mode of transmission, 8 cases (2.31%) by perinatal transmission and 2 cases (0.58%) gave history of blood transfusion.

In CD4 count 90 of 346 cases (26.01%) had CD4 count > 500 cells/ml, 256 of 346 had CD4 count < 500 cells/ml, consisting of 80 (23.12%) with CD4 count between 350-500, 80(23.13%) with CD4 count between 201-350, 58 (16.76%) with CD4 count between 101 -200 and 23 (6.65%) with CD4 count between 51 – 100 and15(4.34%) with CD4count < 50 cells/ml.**Table:2**

The most common clinical presentation was fever (57%), weight loss (45%), oral thrush (35%), cough (15%) and primary generalised lymphadenopathy (10%). **.**Among the HIV positive patients, 135 cases(39.02%) had oral thrush with mean CD4 count 409.97 and candida was grown when the swabs were streaked in SDA, 97(28.03%) of them showed sputum smear positivity for Pulmonary Tuberculosis with the mean CD4 count 173.37. Among the 346 sera screened for Herpes Simplex Virus -2 by IgG ELISA, Toxoplasmosis by IgG ELISA& Cryptococcosis by Latex Agglutination test 50 cases(14.45%) were reactive for HSV-2 with a mean CD4 count of 194.18 cells/ml. , 20 cases(5.78%) were reactive for Toxoplasmosis with a mean CD4 count of 182.35 cells/ml ,12cases(3.41%) were reactive for Cryptococcosis with a mean CD4 count of 96.58 cells/ml. **Table:3**

In our study the most common Opportunistic Infection among the HIV positive patients was Oral Candidiasis followed by Pulmonary Tuberculosis, Herpes Simplex Virus-2, Toxoplasmosis and Cryptococcosis. Ten whole blood samples with CD4 count less than 200 were subjected to HIV-1PCR and all the samples answered positive.

DISCUSSION

Infection with HIV imposes heavy suffering on the affected individuals and in developing countries like India it imposes a great burden on the economy. All the health problems in HIV infection are due to waning immunity and opportunistic infections are significant causes of mortality and morbidity and they pose a great challenge to the era of modern medicine having a great impact on the quality of life in HIV infected individuals. Due to its non curable status, HIV infection continues to be a major health problem. The primary medical care in India for HIV mainly consists of supportive treatment for symptoms, prophylaxis& treatment of Opportunistic infections. So early diagnosis of HIV and its opportunistic infections is vital. This study was done with 346 reactive cases and the prevalence is about 2.8% which is high when compared to the overall prevalence of HIV in Tamil Nadu which is about 0.47%.

In our study majority of the patients were in the age group of 31 to 40 years followed by 41 to 50 years. This observation matches with Nilanjan Chakrabothy et al, ICMR unit, Kolkata has shown that majority of them were in the age group of 31 to 40 years followed by 21-30 years. Aruna Agarwal et al has shown that most of the cases were in the reproductive age group. (11,4).

In our study, majority (60%) are males. This observation matches with studies of Aruna Agarwal et al and Kesav Singh et aland Sarna A et al (4). A majority of the subjects were from rural areas about 77.17% and 22.83% were from urban areas. Similar results were observed by Aruna Agarwal et al and Kesav Singh et al and Sarna A et al. Nilanjan Chakrabothy et al. This shows that awareness about AIDS is very much low in rural areas. (11)

Most of the subjects were educated upto primary level (35.55%) followed by illiterates (32.66%). This indicates that most of the subjects have a low educational status. This is similar to the study conducted by Jayaram et al. In our study 56% of females were illiterate which is in contrast to a study by Gupta et al where only 28% of the females were illiterate.Occupation wise majority were farmers (21.68%) and laborers (20.52%) who may get indulged in risky activities because of illiteracy and lack of awareness. Among the males, 10.4% were drivers whereas in a study by Kesav Singh et al 21.68% were drivers and Vyas N et al has shown that 9.7% were drivers. (15)

Among the females, 88.49% were house wives. Kesav Singh et al have shown that 77.27% were house wives. The distribution according to the marital status has shown that among the males 87.92% were married and 9.18% were unmarried. Among the females 93.53% were married and1.44% were unmarried. This is similar to the study done by Lal et al in North west India but Kesav Singh et al in his study has shown that 76.92% of males and 73.92% of females were married(9).

In our study the major route of transmission is heterosexual 97.11%, followed by

perinatal transmission (2.31%) and blood transfusion (0.58%). Kesav Singh et al has shown heterosexual mode of transmission in 89.33%, perinatal transmission in 21%. Jagdish et al has shown heterosexual mode of transmission in 95.73%, perinatal transmission in3.4%. (7).

Fever(53%), weight loss(50%) followed by oral thrush (39%) and cough(30%) were the most common presenting features in our study which is supported by Aruna Agarwal et al who showed Fever(56%), weight loss(31%) followed by oral thrush (30%) and cough(50%) and Jagdish et al showed Fever(57%), weight loss(45%) followed by cough(18%). (10, 7)

Majority of the patients had CD4 counts > 500, followed by 200 to 500. Only about 30% of the patients had CD4 below 200. In our study CD4 status of the patients reveal that HIV was diagnosed at an earlier stage when the immune system is not much damaged. One more important finding is that the chances of getting opportunistic infection increases when CD4 falls below 200. Akinsen et al has shown that 42.4% had CD4 < 200. (1)

In our study, oral candidiasis is the most common opportunistic infection 135 cases out of the 346 reactive cases (39.02%) had oral thrush. The study of Anant A Takalkar et alwho has shown 39% of oral candidiasis and Aruna et al has shown a prevalence of 24.24% (2,4). Pulmonary Tuberculosis emerged as the second common Opportunistic Infection 97 cases out of 346 cases (28.02%) showed Sputum smear positivity.. But Jagdish et al has shown a prevalence of 25% which matches with our study. TB is highly endemic in India and multi drug resistance is common in HIV (8)

Out of the 346 sera screened for HSV-2 IgG antibodies, 50 were seroreactive(14.45%). The low prevalence of HSV-2 in our study may be due to geographic and socio-economic factors. Nilanjan Chakrabothy et al has shown a prevalence of 47% in his study(12) Genital ulcers are the most common cause of disruption of epithelial barrier and infiltration of CD4 locally resulting easy and early acquisition of HIV.Out of the 346 sera screened for Toxoplasma IgG

antibodies, 20 were reactive (5.78%).The prevalence of Toxoplasmosis in HIV varies from 3 to 97%. Studies have shown that the prevalence of Toxoplasma in USA is 10-40%, in Asia 10-50% (5).

Out of the 346 sera screened for Cryptococcosis by Latex Agglutination Test, 12 were reactive (3.41%). When CD4 count falls below 100, the infection is reactivated and spreads. Usually presents as meningitis and half of them die. The incidence of Cryptococcal meningitis is 3.6% in U.K., 6% in USA and 3% in India. It is a common Opportunistic infection in HIV but remains undiagnosed due to lack of awareness. WHO released "Rapid Advice" guidelines for diagnosis, prophylaxis and management of Cryptococcosis (14). 10 samples were tested by Amplicor HIV-1 DNA PCR a and all the samples(100%)were answered positive. Satarupa Sengupta etal has shown 100% positivity in her study done in Kolkata. The quantification ofHIV-1 DNA by nucleic acid based methods like PCR is necessary for evaluating the efficacy of ART therapy. It is also of great use in diagnosis of HIV in infants and adults in window period (13).

CONCLUSION

This study highlights the epidemiological data and clinical presentation of HIV infection in and around Thanjavur. The role and the complex interrelationship between various social and demographic factors can be understood and thereby the transmission of HIV can be controlled and interrupted. The common age group of affected patients in this study was 31 to 40 years. This data highlights the need of intervention programmes like HIV awareness and safe sex education among the young adults. This study shows increasing trend of HIV infection spread among the house wives which has a direct impact on perinatal transmission and increased number of Paediatric AIDS. This has a powerful influence on the socio economic and cultural development of a country. This study was aimed at providing base line data regarding the common Opportunistic Infections prevalent in our part thereby helps the physicians to take prompt therapeutic measures. Since most patients are diagnosed of HIV only when they present with Opportunistic Infections, a high level of alertness is needed both at the clinical and laboratory level and routine surveillance becomes mandatory. Though curative treatment for HIV is not available at present, we can minimize the spread of HIV infection by early screening and health education.

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TABLE – 1
AGE WISE DISTRIBUTION OF HIV CASES

S.No	Age group	Total	Male	Female
1.	0-10 years	8	5	3
2.	11-20 years	7	5	2
3.	21-30 years	53	29	24
4.	31-40 years	146	73	73
5.	41-50 years	84	61	23
6.	51-60 years	41	29	12
7.	61-70 years	6	4	2
8.	> 70 years	1	1	0

TABLE – 2
CD4 COUNT OF HIV REACTIVE CASES

S.NO.	CD4 COUNT	TOTAL	MALE	FEMALE
	CELLS/ML			
1.	< 50	15	9	6
2.	51-100	23	16	7
3.	101-200	58	37	21
4.	201-350	80	52	28
5.	351-500	80	46	34
6.	> 500	90	47	43

TABLE – 3

CD4 COUNT AMONG HIV CASES WITH OPPURTUNISTIC INFECTIONS

S.NO.	NAMEOF OPPURTUNISTIC INFECTION	TOTAL NUBER OF CASES	PERCENTAGE	MEANCD4 COUNT CELLS/ML
			(N:346 CASES)	
1.	ORAL CANDIDIASIS	135	39.02%	409.97
2.	PULMONARY TUBERCULOSIS	97	28.02%	173.37
3.	HERPES SIMPLEX VIRUS -2	50	14.45%	194.18
4.		20	5.78%	182.35
	TOXOPLASMOSIS			
5.	CRYPTOCOCCOSIS	12	3.41%	96.58

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