Seroprevalence Of HIV In High Risk Group Urban And Rural Population (In Krishna District)

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Abstract : **Objectives**:To study the prevalence of seropositivity among the rural and urban patients who dialysis patients and hemophiliacs in the Krishna district.Methods: The study was conducted on the high risk group 557 individuals who were attending STDclinics, undergoing repeated dialysis, hemophiliacs. Subjects were devided in to 6 Groups. Group-1:urban-Patients attending to STD O.P with history of multiple exposures – 247.Group-2;rural patients attending to STD OP with history of multiple exposures 183.Group-3 urban Patients undergoing repeated dialysis and attending Nephrology clinic – 48. Group - 4 rural patients undergoing repeated dialysis and attending nephrology clinic-25.Group-5 urbanHaemophiliacs taking frequent blood transfusion and / or factors-32.Group- 6 rural hemophiliacs taking frequent blood transfusion and /or factors-22.**Results**: HIV prevelance is more in urban patients(Group-1) who were attending STD clinic with multiple exposures (11.37%) than the rural patients(Group-2) who were attending STD clinic with multiple exposures (9.29%).urban population has significantly high prevalence<0.05 of HIV sero positivity than the rural population. Prevalence of HIV in the hemophiliacs(Group-5&Group-6) and dialysis patients(Group-3&Group-4) were zero in both urban and rural groups. patients attending STD clinic showed the high prevalence<0.001 HIV sero positivity than dialysis patients and hemophiliacs. **Conclusion:** HIV prevalence is more in urban were attending the STD clinics,population who were attending STD clinics.Haemopheliacs and dialysis patients does not show prevalence of HIV. [Sumangali.P et al NJIRM 2013; 4(4) : 51-56]

Key Words: HIV,ELISA,STD,Dialysis,Hemophilia.

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Introduction: Human Immunodeficiency Virus (HIV) is a retrovirus that causes acquired immunodeficiency Syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life threatening opportunistic infections[1.]

One of the earliest documented HIV-1 infections was discovered in a preserved blood sample taken in 1959 from a man from Leopoldville, Belgian Congo . However, it is unknown whether this anonymous person ever developed AIDS and died of its complications.

Another early case was probably detected that same year, 1959[2], in a 48-year-old Haitian, who 30 years before had immigrated to the United States and at the time was working as a shipping clerk for a garment manufacturer in Manhattan. He developed similar symptoms to those just described for the British sailor, and died the same year, apparently of the same very rare kind of pneumonia.

In 1969[3], a 15-year-old African-American male known to medicine as Robert R. died at the St. Louis City Hospital from aggressive Kaposi's sarcoma. AIDS was suspected as early as 1984, and in 1987, researchers at Tulane University School of Medicine onfirmed this, finding HIV-1 in his preserved blood and tissues.

In 1976[4], a Norwegian sailor named Arvid Noe, his wife, and his nine-year-old daughter died of AIDS. The sailor had first presented symptoms in 1969, four years after he had spent time in ports along the West African coastline. Tissue samples from the sailor and his wife were tested in 1988 and found to contain the HIV-1 virus (Group O).

In May 1983[5], doctors from Dr. Luc Montagnier's team at the Pasteur Institute in France, reported that they had isolated a new retrovirus from lymphoid ganglions that they believed was the cause of AIDS. The virus was later named lymphadenopathy-associated virus (LAV) and a sample was sent to the U.S. Centers for Disease Control, which was later passed to the National Cancer Institute (NCI).

In May 1984[6] a team led by Robert Gallo of the United States confirmed the discovery of the virus, but they renamed it human T lymphotropic virus type III (HTLV-III). In January 1985[7] a number of more detailed reports were published concerning LAV and HTLV-III, and by March it was clear that the viruses were the same, were from the same source, and were the etiological agent of AIDS.

In May 1986[8], the International Committee on Taxonomy of Viruses ruled that both names should be dropped and a new name, HIV (Human Immunodeficiency Virus), be used.

HIV emerged later in india than it did in many other Countries, but this has not limited its impact. Infection rates soared throughout the 1990s, and have increased further in recent years. The crisis continues to deepen, as it becomes clearer that the epidemic is affecting all sectors of Indian society, not just the groups, such as sex workers and truck drivers, that it was originally with. W.H.O recorded nearly 20,303 HIV positive cases upto the end of 1985 but none was recorded in india. in the year, 1986 first case of HIV was diagnosed in Chennai, Tamilnadu[9]. In india about 1 72000 patients died of aids related diseases in the year 2009 and also nearly 2400000 patients living with HIV[10].

Enzyme linked immunosorbent assay(ELISA) system was first developed in the early 1960[(11)].the test consists of antibodies bounded to enzymes.the enzymes catalyse a reaction and gives visually discernable end product while reacting with antibodies.antibody binding sites remain free to react with their specific antigens.the formation of coloured end product can be detected by direct observation.In the detection of HIV elisa test is more sensitive than the culture methods.

Material And Methods : The study was conducted on the high risk group 557 individuals that is who were attending STD clinics,undergoing repeated dialysis,hemophiliacs. Subjects were devided in to 6 Groups. Group-1:urban-Patients attending to STD O.P with history of multiple exposures –247
Group-2: rural patients attending to STD OP with history of multiple exposures 183.
Group-3 : urban Patients undergoing repeated dialysis and attending Nephrology clinic – 48
Group 4 rural patients undergoing repeated dialysis and attending nephrology clinic-25
Group-5: urban :Haemophiliacs taking frequent blood transfusion and / or factors-32
Group -6: rural hemophiliacs taking frequent blood transfusion and /or factors-22
Among the 557 patients 3 27 patients were from the urban areas and 230 patients were from rural areas.All the patients were informed about the test procedures and taken consent. Results were analysed by using student t-test and values were expressed as mean and standard deviation. Results were considered as significant at p-value <0.05

Collection of Sample: Following Universal precautions 5 ml of whole blood was collected from each of the selected persons under strict aseptic conditions. Disposable syringes and needles were used.

Separation of Serum : Blood was allowed to clot; the clear serum was transferred into sterile test tubes. It was then centrifuged and the clear supernatant is transferred into small screw capped autoclavable plastic vials (Lax brow vials) for preservation.

Preservation of Serum: Serum was collected in Lax brow vials and preserved at 2-8degrees Celsius in the refrigerator until the test procedure was performed.

Test employed : Microwell (ELISA) test for the detection of antibodies to HIV -1 (Including sub group O & C and HIV – 2 in human serum/plasma[(12)]

Principle: Microwell ELISA HIV test is an enzyme immunoassay based on indirect ELISA

Recombinant proteins gp41, C terminus of gp120 and gp36 for HIV -1 & HIV-2 representing immunodominant epitopes are coated onto microtiter wells. Specimens and Controls were

added to the microtiter wells and incubated. Antibodies to HIV – 1 and HIV – 2 if present in the specimen, will bind to the specific antigens adsorbed onto the surface of the wells.
The plate was then washed to remove unbound material. Horse raddish peroxidase (HRP) Conjugated antihuman IgG was added to each well. This conjugated will bind to HIV antigen – antibody complex present. Finally substrate solution containing chromogen and hydrogen peroxide was added to the wells and incubated. A blue colour will develop in proportion to the amount of HIV -1 and/or HIV-2 antibodies present in the specimen.

The colour reaction was stopped by a stop solution. The enzyme substrate reaction was read by EIA reader for a absorbance at a wavelength of 450 nm. If the sample does not contain HIV-1 of HIV -2 antibodies then enzyme conjugate will not bind and the solution in the wells will be either colorless or only a faint background co lour develops.

The commercial kit used was Microlisa – HIV manufactured by J.Mitra & Co., pvt. Ltd, New Delhi. LOT NO 07046.AP.EXP 06/2007.

Test Procedure :

1) 100 micro liters of sample diluent was added to A-1 well as blank
2) 100 microliters Negative Control in each well no.B-1 & C-1added respectively. Negative Control was ready to use and hence no dilution was required.
3) 100 microliters of Positive Control in D-1, E-1, & F-1 wells wasadded. Positive Control was ready to use and hence no dilution was required.
4) 100 microliters sample diluent in each well was added starting from G-1followed by addition of 10microliters sample. Alternatively transfer 100 micro liter of each sample diluted in sample diluents (1:11), in each well starting from G-1 well.
5) cover seal applied.
6) Incubated at 37`C +/- 2`C for 30 min. +/- 2min.
7) While the samples were under incubation working wash solution prepared and working conjugate as specified in preparation reagents.
8) Plate was taken out from the incubator after the incubation time was over and, wells were washed 5 times with working wash solution according to the wash procedure.
9) 100 microliters of working Conjugate solution was added in each well includingA-1.
10) cover seal was applied
11) Incubated at 37`C +/- 2`C for 30 min. +/- 2 min,
12) Aspirated and washed as described in step no 8.
13) 100 microliters of working substrate solution was added in each well including A-1.
14) Incubated at room temperature (20-30`C) for 30 min. in dark.
15) 50 microliters of stop solution was added.
16) Absorbance read at 450 nm within 30 min. in ELISA READER after blanking A – 1 well.

Quality Control:

Abbreviations

NC - Absorbance of the Negative Control
NCx - Mean Negative control
PC - Absorbance of the Positive Control
PCx - Mean Positive control

Test validity : Blank acceptance criteria:
Blank must be < 0.100 in case of differential filter being used. In case differential filter is not available in the reader the blank value may go higher.
Negative Control Acceptance Criteria: NC must be </= 0.150. If it is not 50, the run is invalid and must be repeated

Positive Control Acceptance Criteria:

1. Positive Control must be >/= 0.50
2. Determine the mean (PCx) value if one of three positive control values is outside of these limits, recalculate PCx based upon the two acceptable Positive Control values.

3. If two of the three positive control values are outside the limits, the assay is invalid and the test must be repeated.

Cut off value:

Absorbance			
NC – 0.042	B1well	PC - 1.412	D1we
0.040	C1 well	1.392	E1we
		1.407	F1 w
Total :	0.082 2 wells	Total: 4.211	3 well
NCx = 0.082/2 = 0.041		PCx = 4.211/3 = 1.4	

The is calculated by adding Mean Negative Control (NCx) and Mean Positive Control (PCx) as calculated above and the sum is divided by 6.

$$\text{Cut off Value} = \frac{NCx + PCx}{6} \qquad NCx = 0.041,\ PCx = 1.403$$

$$\text{Cut off Value} = \frac{0.041 + 1.403 - 1.444}{6\text{cut off value}} = \frac{0.240}{6}$$

Results : Figure 1 shows that patients attending STD clinics have high seroprevelance for HIV .dialysis patients and hemophiliacs doesnot showed seropositivity for HIV.

Figure 2: shows that hiv prevalence is more in urban patients(group-1) who were attending STD clinic with multiple exposures (11.37%)than the rural patients(group-2) who were attending STD clinic with multiple exposures (9.29%).urban population has significantly high prevalence<0.05 of hiv sero positivity than the rural population.

Prevalence of hiv in the hemophiliacs (group-5&group-6) and dialysis patients(group-3&group-4) were zero in the present study (Table 1) in both urban and rural groups. Patients attending STD clinic showed the high prevalence<0.001 HIV sero positivity than dialysis patients and hemophiliacs.

Table 1:Total no of patients ,no of HIV patients,% of prevelance in patients attending STD clinics, dialysis patients ,hemophiliacs.

	Total no of patients	No of HIV +ve cases	% of prevelance
Urban std	247	28	11.37
Rural std	183	17	9.29
Urban dialysis	48	0	0
Rural dialysis	25	0	0
Urban hemophiliacs	32	0	0
Rural hemophiliacs	22	0	0

Figure -1: HIV seroprevelance in std clinics dialysis patients and hemophiliacs.

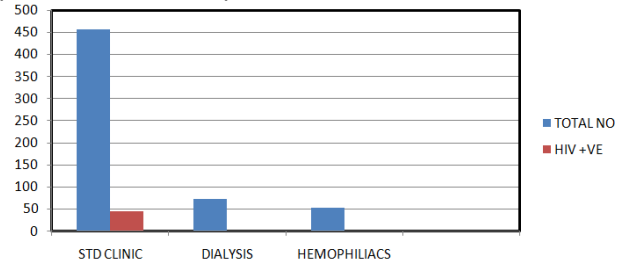
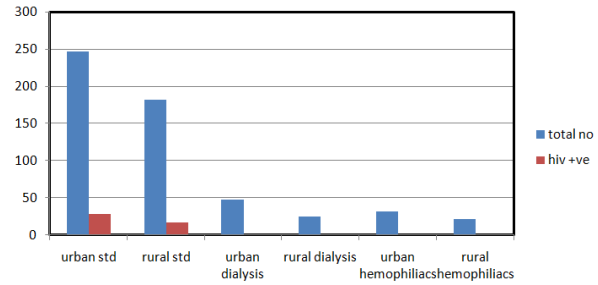


Figure-2: rural urban differences in prevelance of HIV in patients attending STD clinic ,dialysis patients and hemophiliacs.



Discussion: HIV is an lentivirus that produces acquired immune deficiency syndrome(AIDS), that decreases the resistance of the individual and is prone for opportunistic infections.HIV attacks T lymphocytes, macrophages and dendritic cells.

HIV replicate inside human cells.when a virus particle comes into contact with a cell that carries CD4 on its surface the process begins.the virus particle which has spikes on its surface stick to the CD4.The viral envelope fuse with the cell membrane,leaving the envelope behind,the contents of HIV particle are released into cells inside the cell the enzyme reverse transcriptase converts the viral RNA into DNA,which is transported to cell's nucleus ,where it combines with human DNA by the enzyme integrase,known as provirus

The HIV provirus within the cell,when activated,treats HIV genes in same manner as human genes .first,the messenger RNA is formed,which is transportted outside the nucleus which in turn produces new HIV proteins and enzymes.HIV particles are released by budding with active role by the enzyme protease.

The replication process begins again when newly matured HIV particle infect another cell and spreads throught the human boy and the person is infectious. Since the beginning of the Pandemic, three main routes of transmission for HIV have been identified.

- Sexual route: The majority(42%)[13]) of HIV infections are acquired through unprotected Sexual relations.
- Blood or blood products: This transmission route can account for infections in IV Drug users, hemophiliacs transfusion of Blood and blood products and reuse of needles.
- Mother to child transmission (MTCT): This can occur in utero during the last weeks of pregnancy and at child birth.
- In our study it was observed that the STD clinic patients have the higher prevalance of HIV +ve [14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31] The decreased prevalence of HIV in haemopheliacs and dialysis patients may be due to screening methos before the blood transfusion,selection of blood donars i.e.,the preferance given to near relatives and also educating the people regarding HIV.

Conclusions: In this study we conclude that HIV prevalance is more in urban population who were attending STD clinics.Haemopheliacs and dialysis patients does not show prevalence of HIV. We opined that to reduce the HIV in STD clinic patients universal precautions and infection control procedures must be followed.

References:

1. Lippincott's illustrated reviews, microbiology 2 nd ed, page 299, wolters kluwer health India, pvt,ltd..New Delhi.
2. Zhu, T., Korber, B. T., Nahmias, A. J., Hooper, E., Sharp, P. M. and Ho, D. D. (1998). "An African HIV-1 Sequence from 1959 and Implications for the Origin of the Epidemic". *Nature*391 (6667): 594–7.
3. Garry RF, Witte MH, Gottlieb AA, Elvin-Lewis M, Documentation of an AIDS virus infection in the United States in 1968. JAMA, 1988 Oct 14; 260(14):2085-7.
4. Frøland SS, Jenum P, Lindboe CF1983 May 20; 220(4599):868-71. HIV-1 infection in Norwegian family before 1970. lancet. 1988 Jun 11;1(8598):1344-5.
5. Barré-Sinoussi F, Chermann JC, Rey F, Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS), science. 1983 May 20;220(4599):868-71.
6. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science. 1984 May 4; 224(4648):497-500.
7. Marx JL,A virus by any other name Science 22 March 1985: Vol. 227 no. 4693 pp. 1449-1451
8. Coffin J, Haase A, Levy JA, Montagnier L, What to call the AIDS virus? Nature .1986 May 1-7; 321(6065):10.
9. Bureau of hygiene and tropical diseases (1986),aids news letter issue/january 30th
10. NACO annual report 2010-2011;5-6
11. .Rosalyn S. Yalow and Solomon A. Berson, IMMUNOASSAY OF ENDOGENOUS PLASMA INSULIN IN MAN, . J Clin Invest. 1960 July; 39(7): 1157–1175.
12. Bailey & Scott's Diagnostic Microbiology, 12e), Betty A. Forbes PhD D(ABMM) F(AAM), Daniel F. Sahm PhD D(ABMM) F(AAM), Alice S. Weissfeld PhD D(ABMM) F(AAM. ELSEVIER PUBLICATIONS ,PRINTED IN CHINA ,2007
13. Harrison's principles of internal medicine, 16 th ed,page 1079 ,Mc Graw Hill,USA.
14. Solomon S, Anuradha S, Ganapathy M, Jagadeeswari. Sentinel surveillance of HIV-1 infection in Tamilnadu, India.int J STD,1994 Nov – Dec,5(6):445-6
15. Devinder Mohan Thappa, Sowmya Kaimal ,Sexually transmitted infections in India: Current status (except human immunodeficiency virus/acquired

immunodeficiency syndrome) Year : 2007 | Volume : 52 | Issue : 2 | Page : 78-82
16. Mehendale SM, Shepherd ME, Divekar AD, Gangakhedkar RR, Kamble SS, Menon PA, et al. Evidence for high prevalence and rapid transmission of HIV among individuals attending STD clinics in Pune, India. *Indian J Med Res* 1996; 104:327–35.
17. Atul Karkare, Anup R. Anvikar, Vikas G. Rao, Jyothi Bhat, Tapas Chakma, S.R. Qamra
18. V. Gadge, P. Dour, Mahendra Ukey, Canina Luke, Arvind Kavishwar HIV Infection and Sexually TransmittedDiseases in Central India.
19. *Proceeding of National Symposium on Tribal Health.*
20. Jeanette J Rodrigues,, Sanjay M Mehendale, Mary E Shepherd et al, Risk factors for HIV infection in people attending clinics for sexually transmitted diseases in India. *BMJ1995;311:283*
21. Sanjay M. Mehendale, Jeanette J. Rodrigues, Ronald S. Brookmeyer, , Mary E. Shepherd, Ann E. Rompalo, et al Incidence and Predictors of Human Immunodeficiency Virus Type 1 Seroconversion in Patients Attending Sexually Transmitted Disease Clinics in India;Oxford Journals>Medicine>The Journal of Infectious Diseases,Volume 172, Issue 6,Pp. 1486-149
22. Gagandeep Kang, Reuben Samuel,T. S. Vijayakumar, S. Selvi, G. Sridharan, David Brown, Christine Wanke Community prevalence of antibodies to human immunodeficiency virus in rural and urban Vellore, Tamil Nadu, *Natl Med J India* 2005;18:15–17
23. Solomon S, Kumarasamy N, Ganesh AK, Amalraj RE. Prevalence and risk factors of HIV-1 and HIV-2 infection in urban and rural areas in Tamil Nadu, India.int J STD AIDS.1998 Feb; 9(2):98-103.
24. Barongo, Longin R.; Borgdorff, Martien W.; et al. The epidemiology of HIV-1 infection in urban areas, roadside settlements and rural villages in Mwanza Region, Tanzania,official journal of the international aids society, December 1992 - Volume 6 - Issue 12
25. Sheela Godbole & Sanjay Mehendale, Risk factors for HIV infection in people attending clinics for sexually transmitted diseases in India; Indian J Med Res 121, April 2005, pp 356-368.
26. Nasr M. El Sayed, Peter John Gomatos, , Consuelo M. Beck-Sagué, Ursula Dietrich, Hagen von Briesen, Epidemic Transmission of Human Immunodeficiency Virus in Renal Dialysis Centers in Egypt,Oxford Journals>Medicine>The Journal of Infectious Diseases,Volume 181, Issue 1Pp. 91-97.
27. Poignet JL, Desassis JF, Chanton N, Litchinko MB, Zins B, Kolko A, Patte R, Sobel A, Prevalence of HIV infection in dialysis patients: results of a national multicenter study, Nephrologie. 1999;20(3):159-63.
28. M. Velandia, V. Cárdenas, S.K. Fridkin, Transmission of HIV in dialysis centre, *The Lancet*, Volume345,Issue8962,Pages1417-1422
29. MV Ragni, A Winkelstein, L Kingsley, JA Spero, and JH Lewis, 1986 update of HIV seroprevalence, seroconversion, AIDS incidence, and immunologic correlates of HIV infection in patients with hemophilia A and B,Blood, Vol 70. No 3 (September), 1987: pp 786-790
30. Bukurije Zhubi, Ymer Mekaj, Zana Baruti, Ilirijane Bunjaku, Mazllum Belegu, Transfusion-transmitted infections in haemophilia patients. Bosnian journal of basic medical sciences;2009,9(4)271-7
31. Saha D,Agarwal SK, Hepatitis and HIV infection during haemodialysis., Journal of the Indian Medical Association[2001, 99(4):194-9, 203, 213].

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