



Seroprevalence of HIV among Hospital Based Patients around Indore with Research Recommendations

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ABSTRACT: Several useful reviews on the epidemiology of AIDS have been published. Our studies are focused on seroprevalence of HIV among hospital based patient in and around Indore. This study was conducted in the Department of Microbiology, Central Lab Indore, located at Yashvant Plaza in front of Railway station, Indore, with its associated CHL hospital (CHL). CHL is an 800 bedded multi especially tertiary care hospital, located at Nehru Nagar Indore, providing health care services to both rural and urban population of nearly 80 lakh, in and around Indore. A total number of 32534 patients of both gender, visiting the out patients department of Microbiology Central Lab, Indore for seeking medical treatment during the period of four year, that is, from April 2009 to March 2012, were included in the present study. These studies will check out the sensitivity and specificity of ALISA compared to Rapid tests. Research also include various data like retrospective analysis of HIV seropositivity data during April 2008-09 to March 2011-12, revealed that seropositivity is increasing (doubled up), in this geographical area. Studies conclude some recommendations' like educating young person's about HIV before they begin engaging in behaviours that place them at risk for HIV infection at school and collage levels. Studies lighted graphically on distribution of HIV according to age and gender wise, hence it will help to make policies, programmes etc. to government for citizens.

Key words: Hospital, Seroprevalence, HIV, AIDS

INTRODUCTION

In the beginning AIDS was recognized in Africa through the study of patients with the disease, (Clumeck *et al.*, (1983), Offenstadt *et al.*, (1983), Clumeck (1984), Perre *et al.*, (1984), Piot *et al.* (1984)) and the subsequent documentation of infection with the human immunodeficiency virus (HIV) (Bayley *et al.*, (1985), Clumeck *et al.*, (1985). Epidemiologic studies have used case surveillance (Mann *et al.* (1986), Bigger, (1986) and serologic surveys (Mann, *et al.*, (1986), Melbye *et al.* (1986) to show the pattern of heterosexual transmission, (Mann *et al.* (1986) risk factors for acquisition, (Van de Perre *et al.* (1985), Kreiss *et al.* (1986), Van de Perre *et al.*, (1987), Piot *et al.* (1987), Simonsen, *et al.*, (1984), N'Galy *et al.* (1988), and the prevalence of infection. Several useful reviews on the epidemiology of AIDS have been published (Quinn *et al.*, (1986), Mann *et al.*, (1988), Piot and Carael (1988), Piot *et al.*, (1986). Although intensive efforts have rightly focused on preventing further transmission; the large number of cases of AIDS will bring attention back to the clinical problems of

patients with AIDS.

MATERIAL AND METHODS

A total number of 32534 patients of both gender, visiting the out patients department of Microbiology Central Lab, Indore for seeking medical treatment during the period of four year, that is , from April 2009 to March 2012, were included in the present study.

A. Study Groups

Both OPD and admitted patients, who were screened for HIV, constitute our study group.

B. Study Centre

Present study was conducted in the Department of Microbiology, Central Lab Indore, located at Yashvant Plaza in front of Railway station, Indore, with its associated CHL hospital (CHL). CHL is an 800 bedded multi especially tertiary care hospital, located at Nehru nagar Indore, providing health care services to both rural and urban population of nearly 80 lakh, in and around Indore.

C. Collection of Sample

As per the policy laid by the hospital infection control committee (HICC) of CRGH, all patients undergoing any major / minor operative or invasive procedures are routinely screened for HIV, BHsAG and HCV. Also patients with high risk group, such as, those visiting STD clinic, and those suffering from TB, were also screened or HIV/ HBsAg /HCV were included in the present study. Three CC blood was collected from antecubital vein in clean, plain vials, with proper antisepsis of the skin. All samples were allowed to clot and clear, non-haemolysed, non lipaemic serum samples were collected in clean vials. All patient demographic data including name, age, sex, address, OPD / IPD no. and that of the samples collection etc. All serum samples were checked by ELISA and rapid test for HIV, within 24 hours of collection.

D. Separation of Serum Samples

The important step after the collection of sample is the serum separation from the collection blood. Generally in these cases the blood samples were collected a day before and were kept in the refrigerator and were allowed to settle down, and after a period of 24 hours. Mostly the separated serum was seen clearly and was taken in the required amount with the help of the pipette, but in certain cases where serum separation was not successfully by this method another method was used that is as follows:

Centrifugation. The centrifugation technique basically works on the principle of settlement of the heavier particles down and the lighter particles or the lighter matter is above and from there it is easily separated. The similar process was followed in the test tubes, the test samples, which were to be centrifuged, were taken and the balance was deliberately maintained by using test tubes with filled water and the RPM (1900 RPM) and the time was also set (2 min.).

1. Rapid Test. Retro quick HIV is a membrane based flow through immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum and plasma. Highly purified synthetic peptides of gp 41 and 36, corresponding to the immunodominant regions of HIV-1 and HIV-2 utilised in the test system assist in visual, qualitative, simultaneous detection of antibodies to HIV-1 and HIV-2.

Principle. Retro quick HIV test comprises of a test device striped with distinct band of purified gp120 and gp41 synthetic peptide to HIV-1 and gp36 synthetic peptide specific to HIV-2. At test region '2' the third band striped at region 'c' corresponds to the assay performance control. First the membrane assembly is hydrated with wash buffer and then the specimen is added. Antibodies to HIV-1 and /or 2 if present are captured by the respective antigens, after washing with buffer, with protein a conjugated gold sol reagent is added to reveal the presence and absence of bound

antibodies. Post final wash a positive reaction is visualized by the appearance of purple coloured bands at the last region '1' and '2' is a negative test result. The band serves appearance of control to validate sample addition, reagent and assay performance.

Reagents and Materials Supplied:

1. Ready to use individually pouched, flow through test devices striped with HIV 1 specific purified synthetic peptides at test region '1' and HIV '2' specific purified synthetic peptides at test region '2' and a blue dyed protein a based control band a region 'c' along with a specimen dropper and desiccant.
2. Dropper bottle with ready buffer solution.
3. Dropper bottle ready to use protein a conjugate gold solution.
4. Package insert.

(a) Test Procedure (Microlisa - HIV Microwell ELISA)

1. Bring all reagent and specimen to room temperature (25-30) before use.
2. Tear open the soil pouches and retrieve the required of retroquick HIV membrane test device and label appropriately.
3. Add two drop of wash buffer into the reaction port of the device and allow soak through completely.
4. Use the sample dropper provided add one drop of the serum plasma (25microlitre) specimen into the reaction port. Allow to soak through completely.
5. Add 3 drops of wash buffer to the reaction port and allow soak through completely.
6. Add 2 drops of wash buffer and allow the wash buffer to soak through completely.
7. Read and record the result immediately.

Interpretation of Results

Positive

Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).

Negative

One red line appears in the control region (C) no apparent red or pink line appears in the test region (T).

Invalid

Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

2. ELISA Test

ELISA is the most commonly performed screening test. Screening assay must detect all positive sera that should be highly sensitive even if some false positives do occurs. However result of a screening test could never be used as the final interpretation of HIV status. And individual identified on the basis of 1 screening assay as technical errors can occur. The serum reactive in screening assay is subjected to confirmatory test (as per policy and strategy) of testing to be classified as reactive only if is reactive in repeated assay.

Material Required

ELISA kit and Serum etc.

Components in Each MICROLISA HIV Kit

Store all components at 2-8 °C when not in use. Expiry date on the kit indicates that beyond which the kit should not be used.

1. Micro Lisa – HIV Strip plates – 12 strips (12x8 wells)
2. Sample diluent-1 bottle (20ml).
3. Enzyme conjugate concentrate (100x) 1 vial (0.25ml)
4. Conjugate diluent _ 1 bottle (15ml)
5. Wash buffer concentrate (25x) – 1 bottle (50ml) (PBS with surfactant, Dilute 1:254 with distilled water before use).
6. TMB Concentrate (100x) – 1 vial (0.25ml)
7. Substrate (TMB diluent) – 1 bottle (20ml)
8. Control - !- 1 vial (2.0ml)
(Ready to use normal human serum; negative for HIV, HCV and HBsAg)
9. Control - !- 1 vial (2.0ml) (Ready to use, inactivated and diluted human serum; positive for HIV antibodies)
10. Stop solution – 1 vial (6ml) (Ready to use, 2 N sulphuric acid)
11. Plate sealers– Adhesive baked sheets for sealing micro well plate/strips

Preparation of Reagents

The following reagents were prepared before or during assay procedures. Reagents and samples were kept at room temperature (20-30 °C) before beginning the assay and left at room temperature during testing. After use return reagents to 2-8 °C all containers used for preparation of reagents were cleaned thoroughly and rinsed with distilled or deionized water. The incubator was pre-warmed at 37 °C.

Sample Preparation**1. Tube Dilution**

The tubes were marked carefully for the proper identification of the samples. The serum samples were diluted to be tested, with sample diluent (1:11 dilution) in separate tubes (20µl diluent + 20µl sample). A separate tip was used for each sample and then discarded as biohazard waste.

2. Micro Well Dilution

- (a) 100µl of sample diluent was pipette into the micro well.
- (b) 10 µl of serum sample to be tested was added.
- (c) Ensure thorough mixing of the sample to be tested.

3. Preparation of Wash Buffer

(a) The buffer concentration was checked for the presence of salt crystals. The crystals resolubilized by warming at 37°C until all crystals dissolved, if the crystals were present in the solution.

(b) 50 ml of buffer was prepared (2 ml concentrate buffer with 48ml. water) for each micro Lisa strip used and was mixed well before use.

(c) 20 ml. 25 x wash buffer concentrated was mixed with 480 ml. of deionizer water. Wash buffer is stable for 2 months when stored at 2-8 °C.

4. Preparation of Working Conjugate

Conjugate concentrated was diluted by 1:100 in conjugated diluent, working conjugate was not stored. A fresh dilution for each assay in a clean glass vessel was prepared.

5. Preparation of Working Substrate Solution

TMB concentrate was diluted by 1: 100 in substrate TMB diluent. The 100X solution was crystallized during storage. Crystals were checked before use, if crystals were present, solubilize by warming at room temperature.

Test Procedure

Once the assay has started, complete the procedure without interruption. All the reagents should be dispensed in the centre of the well and the tip of the pipette should not touch the wall of the micro well.

Fit the strip holder with the required number of MICROLISA-HIV strips. The sequence of the procedure must be carefully followed. Arrange the assay control wells so that well A-1 is the reagent blank. From well A-1 arrange all controls in a horizontal or vertical configuration. Configuration is dependent upon reader software.

1. Add 100 µl sample diluent to A-1 well as blank.
2. Add 100µl Negative Control in each well no. B-1 & C-1 respectively. Negative Control is ready to use and hence no dilution is required.
3. Add 100µl Positive Control in D-1, E-1 & F-1 wells. Positive Control is ready to use and hence no dilution is required.
4. Add 100 µl sample diluent in each well starting from G-1 followed by addition of 10µl sample. (Refer MICROWELL DILUTION) Alternatively Transfer 100 µl of each sample diluted in sample diluent (1:11), in each well starting from G1 well. (Refer TUBE DILUTION).
5. Apply cover seal.
6. Incubate at 37°C + 20C for 30 min. + 2 min.
7. While the samples are incubating, prepare Working Wash Solution and Working Conjugate as specified in Preparation of Reagents.
8. Take out the plate from the incubator after the incubation time is over and, wash the wells 5 times with Working Wash solution according to the wash procedure given in the previous section (wash procedure).

9. Add 100 µl of Working Conjugate Solution in each well including A-1.
10. Apply cover seal.
11. Incubate at 37°C + 20C for 30 min. + 2 min.
12. Aspirate and wash as described in step no. 8.
13. Add 100 µl of working substrate solution in each well including A-1.
14. Incubate at room temperature (20 - 30°C) for 30 min. in dark.
15. Add 100 µl of stop solution.
16. Read absorbance at 450 nm within 30 minutes in ELISA READER after blanking A-1 well. (Bichromatic absorbance measurement with a reference wavelength 600 - 650 nm is recommended when available).

CALCULATION OF RESULTS

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 so, the run is invalid and must be repeated.

Positive Control Acceptance Criteria:

1. PC 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 B1 Well	PC - 1.412 D1 Well
- 0.040 C1 Well	- 1.392 E1 Well
Total: 0.082 2 Wells	- 1.407 F1 Well
	Total: 4.211 3 Wells
$NCx = 0.082/2 = 0.041$ $PCx = 4.211/3 = 1.403$	

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

$$\begin{aligned}
 & \frac{NCx + PCx}{6} = \frac{0.041 + 1.403}{6} \\
 \text{Cut off Value} &= \frac{0.041 + 1.403}{6} = \frac{1.444}{6} = 0.240
 \end{aligned}$$

RESULTS AND DISCUSSION

1. Test specimens with absorbance value less than the cut off value are non-reactive and may be considered as negative for anti-HIV.
2. Test specimens with absorbance value greater than or equal to the cut off value are reactive for anti-HIV by MICROLISA-HIV.
3. The O. D. for Crystal clear negative samples can be in minus. However, the minus (-) O.D. does not in any way affect the result interpretation. It rather gives better specificity.

Observations

Observations of present study are as followings:
The highest number of patients (8991) tested for HIV was in the year 2009-10. The HIV reactivity is found to be gradually increasing from 0.83% to 1.70%, during the four years period from 2008-09 to 2011-12.

Table 1: Showing year wise HIV Seropositivity among Hospital based patients.

No.	Year(April to March)	No. of Patients	No. of HIV Reactive	%
1.	2008-09	6952	58	0.83
2.	2009-10	8991	88	0.97
3.	2010-11	8274	112	1.35
4.	2011-12	8317	142	1.70

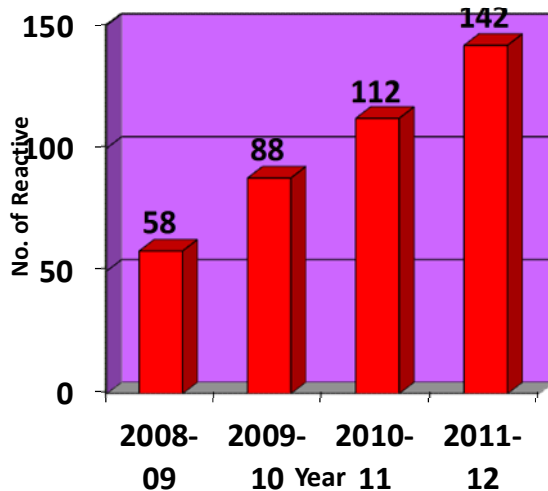


Fig. 1. Number of reactive increasing with year.

Table 2: Showing Gender wise distribution of HIV seropositivity among patients (Year 2008-09 to 2011-12).

Year(April to March)	Total No. of Tested	Total No. of Male Tested	Total No. of Female Tested	Total No. of Reactive	Male Reactive	Female Reactive
2008-09	6952	4518	2434	58	34	24
2009-10	8991	5484	3507	88	60	28
2010-11	8274	3888	4383	112	80	32
2011-12	8317	4408	3909	142	88	54
Total	32534	18298	14239	400	262	138

Out of 32534 patients screened for HIV during four years, 18298 were males and remaining 14239 were females. Out of 18298 males tested, 262 were found reactive and of 14239 females tested, 138 were found reactive for HIV.

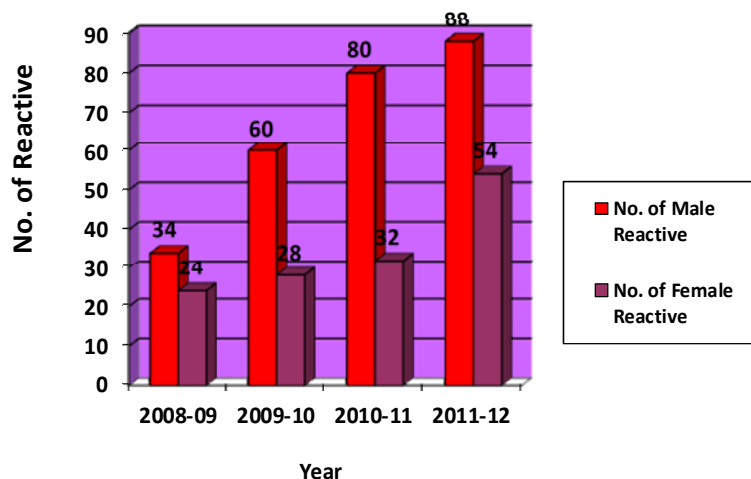


Fig. 2. Gender wise distribution of HIV Reactive Patients (From April 2008-09 to March 2011-12).

Table 3: Age wise Distribution of HIV Seropositivity in Male and Female Patients.

Month	AGE GROUPS													
	1-10 year		11-20 year		21-30 year		31-40 year		41-50 year		51-60 year		60 above	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
April	-	-	-	-	2	4	-	-	-	-	-	-	-	-
May	-	-	-	-	-	6	2	-	2	-	-	-	-	-
June	-	-	-	-	-	-	-	-	2	-	2	-	-	-
July	-	2	-	-	4	2	2	2	-	-	-	-	-	-
August	-	-	-	-	2	4	2	-	-	2	-	-	2	-
September	4	-	2	-	2	-	4	2	4	-	2	-	2	-
October	-	-	2	-	2	2	2	4	-	2	-	-	-	-
November	-	-	2	-	4	2	-	2	2	-	-	-	-	-
December	-	-	-	2	6	2	4	2	-	2	4	-	-	-
January	-	-	-	-	-	4	4	-	2	-	-	-	2	-
February	-	-	-	-	2	-	-	-	-	-	-	-	-	-
March	2	-	-	-	2	2	2	-	2	2	-	2	-	-
Total	6	2	6	2	26	28	22	12	14	8	8	2	6	0

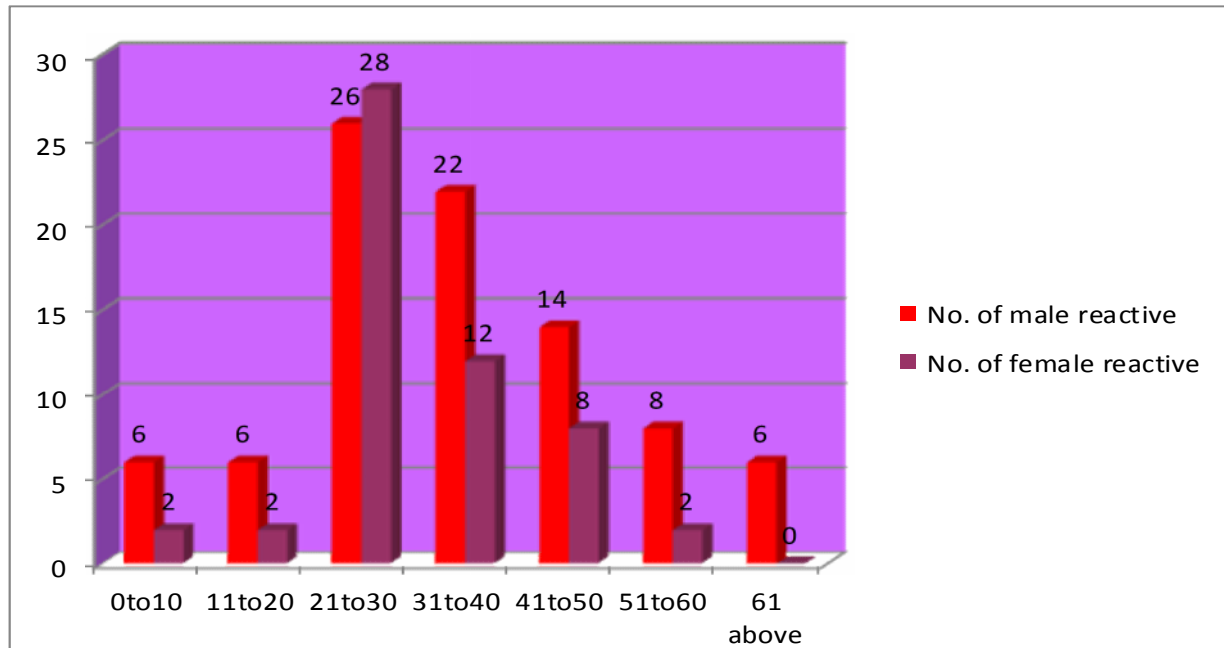


Fig. 3. Number of male and female in different age group.

Table 4: Showing Comparative Evaluation of HIV testing by ELISA and RAPID Test.

Test	Positive	Negative	Total
ELISA	400	32134	32534
RAPID TEST	410	32144	32534

Out of 32534 patients tested for HIV 400 were Reactive by ELISA as compared to 410 by Rapid Test, while 32134 were found Non-Reactive by ELISA in compared with by Rapid Test 32144.

CONCLUSION

A total of 32534 patients of both genders and various age groups ranging from 1 year to 60 years above visiting to Central Lab Indore were tested for HIV by two different methods namely Rapid and ELISA test during a period of four years i.e. from April 2008-09 to March 2011-12, conclusions of the present study are as

follows:

1. Out of 32534 patient tested, 400 were found Reactive for HIV, contributing to 1.22 % seropositivity.
2. Among the HIV Reactive patient 1.43 % were male and 0.96 % were females.
3. HIV seropositivity was found more in males as compared to females. It is evident from the present study that, the control and preventive measures should be aimed at 21-30 years group, that is young sexually age group. More emphasis needs to be given on health education not only in schools and collages but there is a need to reach up to the illiterate, class of the society including the rural population of this region.
4. Retrospective analysis of HIV seropositivity data among hospital based patients population, during April 2008-09 to March 2011-12, revealed that seropositivity is increasing (doubled up), in this geographical area.
5. Both the testing methods used in the present study namely the Rapid test and ELISA test possess a very high sensitivity, and PPV and NPV for detecting HIV seropositivity in patients.
6. However a comparative evaluation of both these tests showed that, ELISA has slightly (marginally) better sensitivity and specificity so also, PPV and NPV than the rapid test.
7. Hence, it is recommended that, ELISA should be preferred over rapid test for screening of HIV seropositivity routinely and Rapid test should be reserved for emergency testing only because of its obvious advantages such as case of performance and visual interpretation.
8. The gradually increasing HIV seropositivity in this group of population is alarming and appropriate measures are required for the effective control and prevention of this disease is ideal.
9. Highest seropositivity has been found among males and females of 21-30 age group followed by 31-40 age groups and the lowest seropositivity is found in 60 above age group.

RECOMMENDATIONS

Research recommendations are following:

- (i) In 2012 numbers of HIV patients have been have been doubled as compared to 2009, so it is must to diagnosis and treat to patients.
- (ii) Distribution of HIV is mainly found in the age group of 21-30 years patients, so it is must to educate youngsters about sex. It is must to arrange interesting programmes at collage level.
- (iii) Posters, advertisements are not sufficient to control HIV. Programmes should also include rapid tests for HIV, or other quick methods to diagnose HIV among the public, where programmes have done.

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