

Primary HIV Infection in Patients Presenting with Conventional Sexually Transmitted Infections (STIs) in Ethiopia: Magnitude and Risk Factors

By

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Acronyms

AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
BED-EIA	HIV Subtypes B, E, and D Enzyme Immunoassay
CDC	Centers for Disease Control and Prevention
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme Linked Immunosorbent Assay
ESTC	Ethiopian Science and Technology Commission
GUS	Genital Ulcers Disease Syndrome
HIV	Human Immunodeficiency Virus
HSV2	Herpes Simplex Virus type 2
LCR	Ligase Chain Reaction
MOH	Ministry of Health
NERC	National Ethical Review Committee
OD	Optical Density
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PHI	Primary HIV Infection
PIHCT	Provider Initiated HIV Counseling and Testing
RECC	Research and Ethical Clearance Committee
RNA	Ribonucleic Acid
SNNPR	Southern Nations and Nationalities Peoples Region
SOPs	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
STARHS	Serologic Testing Algorithms for Recent HIV Seroconversion
STI	Sexually Transmitted Infections
UDS	Urethral Discharge Syndrome
UNAIDS	Joint United Nations Program on HIV/AIDS
VCT	Voluntary Counseling and Testing
VDS	Vaginal Discharge Syndrome
WHO	World Health Organization

Abstract

Inclusion of incidence data in the surveillance system of HIV is important in order to generate accurate data for effective prevention and control of the pandemic. Retrospective cross-sectional HIV seroprevalence and seroincidence survey was conducted to determine the magnitude of Primary HIV Infection (PHI) and, to identify and describe factors associated with it on stored sera of 455 STIs cases that had been collected and stored during the validation study of syndromic case management algorithms for STIs in Ethiopia, between January - May 2003. Standard HIV-antibody tests were done on all sera and IgG-capture BED-EIA on positive samples for detecting recent infections. Negative Serum samples for HIV antibodies were screened by nucleic acid amplification (HIV RNA PCR) for viral RNA. Incidence was calculated using the consensus formula. OR with 95% CI was used to measure the degree of association between associated factors and PHI and, logistic regression analysis was done to identify predictors of PHI.

The overall HIV1/2 seropositivity was found to be 33% and the rate varies among the major STI syndromes, namely, VDS(35%), UDS(20.2%) and GUS(60.5%). Out of the 150 HIV antibody positive cases 15 (prevalence, 3.3%) were recently infected and, annual HIV incidence was estimated to be 11.8% (95% CI, 6.02, 17.58). Acute HIV infection was detected in 7 cases (prevalence, 1.5%) out of 305 HIV antibody negative sera indicating that standard HIV-antibody tests detected only 95.5% of the total HIV infections. The over all prevalence of PHI was 4.8%. Considering both recent and acute infections annual HIV incidence was estimated to be 10.1% (95% CI, 5.13 - 14.97).

Diagnosis of syphilis was found to be an independent risk factor for PHI for both sexes. Age group 35 – 39 years in males was found to be significantly associated with PHI.

The estimated high incidence (10.1%) of HIV-1 infection in this study population indicated that HIV infection is still spreading and targeted intervention is highly recommended. Strong and sustained Provider Initiated HIV Counseling and Testing (PIHCT) with subsequent follow up is recommended for STI patients, particularly those with GUS, during their first appearance at health institutions.

I. Statement of the Problem

The rapid expansion of HIV/AIDS in sub-Saharan African countries has a profound impact on the health sector as well as the socio-economic development of the region in general. In the worst affected countries, the pandemic continues to negatively influence the developmental gains of the past few decades. Despite the concerted efforts to control the epidemic in the last few years, the corresponding global outcome of curtailing the epidemic is not to the extent that averts the epidemic and it seems that the pandemic is continuing unabated in many parts of the world and is still claiming millions of lives. Even if the pandemic is brought under control, we will still continue to wrestle with the after effects for many years to come.

Accurate surveillance data provides essential information in guiding rational HIV control and intervention programs, as well as in monitoring trends of the epidemic. Surveillance data currently being used in Ethiopia rely on prevalence studies but prevalence studies fail to identify those infected recently. Since incidence data depict the current dynamics and trend of the epidemic and also is needed to prioritize resources for prevention and care, it is of paramount importance to include incidence studies as part of surveillance programs. Traditionally, incidence data have been derived from longitudinal cohort studies. However, such types of studies are technically demanding, expensive and subject to bias due to the need for recruitment of a large number of seronegative volunteers and follow up for a longer period of time.

Until recently, a handful of methods including back-calculation from AIDS case reports have been used to estimate HIV incidence. These methods have never provided a complete picture of the HIV epidemic; at most, they provide estimates for specific segments of the population or for people who voluntarily test for HIV. While it has long been clear that information on incident infections is important, it has only recently, with the availability of Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) testing, become feasible to estimate HIV incidence using cross-sectional study designs and blood specimen collection in a community by testing a single HIV- positive specimen. (1, 2, 3)

Detection of contagious, acutely infected persons creates new opportunity for HIV surveillance and prevention. By targeting research efforts on those individuals who are newly diagnosed, relevant information concerning changing behaviors and attitudes can be evaluated. By evaluating how new infections are occurring, who is becoming infected, and whether newly infected persons are receiving referrals and appropriate services, it would be possible to obtain crucial scientific information for resource prioritization and make concerted efforts in the prevention and control of HIV/AIDS pandemic. (1)

II. Background and Literature Review

1. Global and Ethiopian HIV/AIDS Situation

The HIV pandemic continues to spread with about 40.3 million people living with HIV worldwide. Globally, approximately 4.9 million new infections are believed to have occurred in the year 2005, corresponding to about 13,500 new infections

per day. About 3.1 million people have died during the same time period; more than half a million (570,000) were children. (4)

Sub-Saharan Africa has just over 10% of the world's population, but is home to more than 60% of all people living with HIV – some 25.8 million (range: 23.8-28.9 million) in 2005 compared to 24.9 million (range: 23.0-27.9 million) in 2003. In 2005 alone, an estimated 3.2 million people became newly infected, while 2.4 million people died of AIDS, which was 77% of deaths globally. (4)

According to AIDS in Ethiopia fifth report 2004 (5), the 2003 estimate of cumulative number of people living with AIDS was about 1.5 million (3.8% male and 5% female; 12.6% urban and 2.6% rural) out of which about 96,000 were children under 15 years. There were also estimates of 197,000 new infections, 98,000 AIDS cases (46% male and 54% female) and 90,000 AIDS deaths in the adult population in 2003. A total of 128,000 HIV-positive pregnancies and estimated 35,000 HIV-positive live births occurred. Among children aged 0-14 years, there were 35,000 new HIV infections, 25,000 new AIDS cases and 25,000 AIDS deaths. A total of 4.6 million children under 17 years in the country were estimated to be orphaned for different reasons, of which 537,000 were due to AIDS. The estimated national adult incidence showed a steady increase since 1982 and that of 2003 was estimated to be about 0.55%.

HIV/AIDS surveillance activities based on Antenatal Care (ANC) sentinel sites began in 1989 and, based on the available data, since then the HIV epidemic appears to have steadily increased in Ethiopia. The trend of the HIV epidemic from 1982 till 2003 suggests three key points: a continuing gradual rise in

national prevalence (3.3% for 1995, 4.1% for 2001, 4.2% for 2002 and 4.4% for 2003); an urban epidemic that has peaked and plateaued at higher prevalence levels; and a very gradual but steady rise in HIV prevalence in rural Ethiopia. (5)

2. HIV/AIDS and Other Conventional Sexually Transmitted Infections

2.1 Global Situation

Sexually transmitted infections (STIs), by their own right, are major global causes of acute illness, infertility, long-term disability and death, with severe medical and psychological consequences for millions of men, women and infants. WHO estimated that 340 million new cases of STIs have occurred throughout the world in 1999 in men and women aged 15-49 years (6).

There is tangible scientific evidence that a person with an untreated sexually transmitted infections (STIs), particularly involving ulcers or discharge, is at an increased risk of passing on or acquiring HIV during sex. The presence of an STI means that there is more chance of broken skin or membranes allowing the virus to enter or leave the body. The very same cells that the virus is seeking to infect will be concentrated at the site of the STI because these cells are fighting the infection. According to current thinking, the risk of becoming HIV infected from a single exposure is increased 10 to 300 fold in the presence of genital ulcer caused by syphilis, chancroid or genital herpes (HSV-2) (7)

The notion of co-transmission of STI pathogens suggests one location where acute HIV infection might be best detected: STI clinics. Recent studies, using molecular techniques for detecting viral RNA, have shown that STI clinic attendees in the US (8), Malawi (9) and India (10) can have a surprisingly high

prevalence of acute HIV infection. Cameron et al. also generated compelling evidence for co-transmission of herpes simplex virus and HIV in the late 1980s (11). The prevalence of acute HIV infection in the Malawi STI clinic, screened by HIV RNA PCR using a highly specific pooling/resolution-testing algorithm, was found to be 5% (9). In the Indian study done in STI clinic, it was found that 1.5% of antibody-negative specimens collected were positive for HIV p24 antigen (12). In another study (13), most of the persons with acute infections (16 of 23, or 70 percent) were identified by nucleic acid amplification at sexually transmitted disease clinics. The remainder of the acute infections was detected at freestanding HIV testing sites and at jails.

2.2 Ethiopian STIs and HIV Sero-surveillance Situation

Currently, there is no systematic collection of surveillance data of STIs in Ethiopia, apart from routine syphilis tests for pregnant women in antenatal clinics. STIs have increasingly been recognized among certain risk groups, such as commercial sex workers (14, 15, 16). STI prevention and control activities have been neglected until recently that Centers for Disease Prevention and Control (CDC) - Ethiopia is undertaking the program on expansion of HIV/AIDS, STI and TB Surveillance and expansion of laboratory services in Ethiopia as of 2003. Validation of the national STIs syndromic management algorithms and development of the national STIs syndromic management guideline and also development of the national training guideline on syndromic management of STIs were some of the major activities done so far regarding STIs (17).

HIV seroprevalence rate was 12% among patients treated for various types of STIs in 1991 (18) and has increased to 64% in 2001 in patients presented with genital ulcer diseases (19). Moreover, a substantial increase in the association between genital ulcer diseases and HIV has been noted indicating that HIV seroprevalence has increased from 16% (18) to 90% among patients presented with genital ulcer diseases (19). Other recent studies conducted in Ethiopia showed that HSV-2 seroprevalence is significantly higher, at 61.5%, among HIV positive subjects than 29.1% among HIV uninfected persons (20) and, as high as 87% among patients presenting with STIs (21). Having genital ulcer was found to be the most important independent risk factor associated with treatment failure following syndromic treatment algorithm (19) and treatment failure was associated with persistent genital shedding of HIV-1 (22).

Currently, HIV sero-surveillance is based on ANC surveillance system that included 66 sentinel sites (37 urban and 29 rural) and this system continued to be the main source of data. Recently, other source of information such as routine AIDS case reporting sites and service related HIV data sources like blood donors, VCT clients and visa applicants are included in the surveillance system there by increasing coverage to better assess the extent of the pandemic. But, these testing sites do not include tests for recent and acute infections that would help to see the current dynamics of the infection. Systematic STI surveillance is none existent except routine syphilis test being done at ANC site that showed the current national prevalence of 1.8% (5). Other STIs are routinely being reported to MOH but should be included in a systematic surveillance system so that it

would serve as a second generation HIV surveillance data source thereby increasing the accuracy of the data.

3. Recent Developments in Estimation of HIV Incidence and its Importance

Disease stage is strongly associated with individual infectiousness in HIV transmission. Higher viral loads correlate with higher transmission probability in patients with advanced disease (23), and the most striking elevations in HIV viremia and genital-fluid shedding also occur early in acute infection (24, 25). Based on a probabilistic model of the relationship between semen viral load and probability of HIV transmission (26), increases in semen HIV load during acute HIV infection appears sufficient to account for an eight- to 20-fold increase in the odds of transmission per coital act (24).

Since routine HIV antibody tests yield negative results during the first four to five weeks of HIV infection (27), acute infections can be diagnosed during this period only with the use of tests for viral antigens, nucleic acids, or both. Sensitive nucleic acid amplification tests are routinely used by blood banks to protect the blood supply (28). However, concerns about cost and specificity have precluded the use of these tests for clinical testing for HIV, except in the evaluation of suspected acute retroviral syndromes (29).

Recent developments to detect and distinguish recent and long-term HIV-1 infections using laboratory tests have made the measurement of HIV-1 incidence comparatively realistic and practical. The sensitive/less-sensitive testing strategy provided simple laboratory tools to detect recent seroconversion in a cross-

sectional population. These assays are based on differences in antibody titers in recent versus long-term infections and have been used for sometime for estimating population incidence. However, recent work demonstrated limitations of this approach which included subtype-dependent performance and significant variability of "window periods", precluding its use in many areas of the world. Recently an IgG-Capture BED-EIA was developed which detects the increasing level of HIV-IgG as proportion of total IgG following seroconversion and can be used to detect recent seroconversion. The format of the assay, which includes a multi-subtype derived antigen, allows high consistency and similar "window periods" in different subtypes, (30).

Recognition of recent or acute HIV infection is important for several reasons. First, acute HIV infection provides a unique view of HIV transmission and pathogenesis, including early host-virus interactions that require further study. Second, prevention strategies directed at subjects with acute HIV infection may have great impact. Because, during acute infection the large amounts of HIV present in the blood and genital fluids mean that the risk of transmission is higher than later in the course of infection. Third, very early recognition may allow for HIV treatment that could alter the natural history of disease, or even eliminate infection (a condition which is still under clinical trial) (24).

Although HIV-SIT interaction studies in Ethiopia are scarce, a number of international studies revealed that STIs contribute in fueling the HIV epidemic in the world and; the fact that holds true in Ethiopia too. Data on and a clear understanding of HIV incidence (the rate of new infections) in a known risk

groups, such as STI patients, for HIV transmission/acquisition is important for planning targeted interventions to fight the epidemic of HIV/AIDS in Ethiopia.

III. Objectives

1. General Objective

To assess the magnitude and risk factors associated with primary HIV infection among patients presenting with conventional STIs in Ethiopia

2. Specific Objectives

- To determine the prevalence of HIV infection among STI patients.
- To determine the magnitude of primary HIV infection among STI patients.
- To identify and describe the socio-demographic, behavioral and other factors associated with primary HIV infection among STI patients.

IV. Methodology

1. Study Design

The study design was a retrospective cross-sectional HIV seroprevalence and seroincidence survey using standard HIV antibody serologic tests, IgG-capture BED-EIA and HIV RNA PCR by pooling method on stored sera. Serum samples have been collected and stored during a multi-center cross-sectional survey conducted in eight health centers located in Addis Ababa City Administration and SNNPR to validate the performance of syndromic case management algorithms for STIs (17). The validation study was a collaborative research between Ethiopian Health and Nutrition Research Institute (EHNRI), Federal Ministry of Health, and Center for Disease Prevention and Control (CDC) and conducted between January and May 2003.

2. Study Population

The study included a total of 455 consecutive and consenting male and female patients aged 15-49 years presented with genital ulcers, males 15-49 years presented with urethral discharge/dysuria and females presented with vaginal discharge with/without lower abdominal pain. Aliquots of sera collected for syphilis tests and stored at -70 degree centigrade at EHNRI laboratory were used for the study. Data on socio demographic characteristics, clinical history and behavioral factors were collected during the validation study using a structured and pre-tested questionnaire, and these data were used for identifying and describing risk factors associated with primary HIV infections.

3. Sample Size

With regards to sample size, 455 STIs cases' data and their corresponding stored sera were considered to be enough for the study. Because of the fact that STI patients of the validation study were consecutive and consenting (not randomly selected), representativeness of the sample was not of much concern. That was also characteristic of laboratory-based studies in health facility set-ups. However, considering random selection, and taking total population of about 5 million, degree of precision of 2%, the estimated Malawi incidence of HIV-1 infection in male patients with STIs (because there was no such study in Ethiopia) of 5.0% and 95% confidence level, the sample size was calculated to be 456, which was almost equal to the number of stored sera (455).

4. Laboratory Procedures

4.1 Standard HIV Antibody Testing

Serum samples were screened with a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit for identification of HIV1/2 antibodies (Vironostika HIV Uni-Form II plus O, biomerieux, Boxtel, Netherlands). Specimens tested positive with the first ELISA, were again tested with the second ELISA (Murex HIV 1.2.0, Dart Ford, UK). Discrepant results with the above tests were confirmed by HIV1/2 Western blot (Genlabs diagnostics, Singapore).

4.2 Primary HIV Infection (PHI) Determination

PHI is the proportion of individuals who are HIV antibody positive and infected recently and those who are HIV antibody negative, but HIV RNA positive. BED HIV-1 incidence assay was used to identify individuals with recent HIV infection and HIV RNA PCR assay by pooling was used to identify those individuals who were HIV antibody negative, but HIV RNA positive.

4.2.1 Incidence Assay

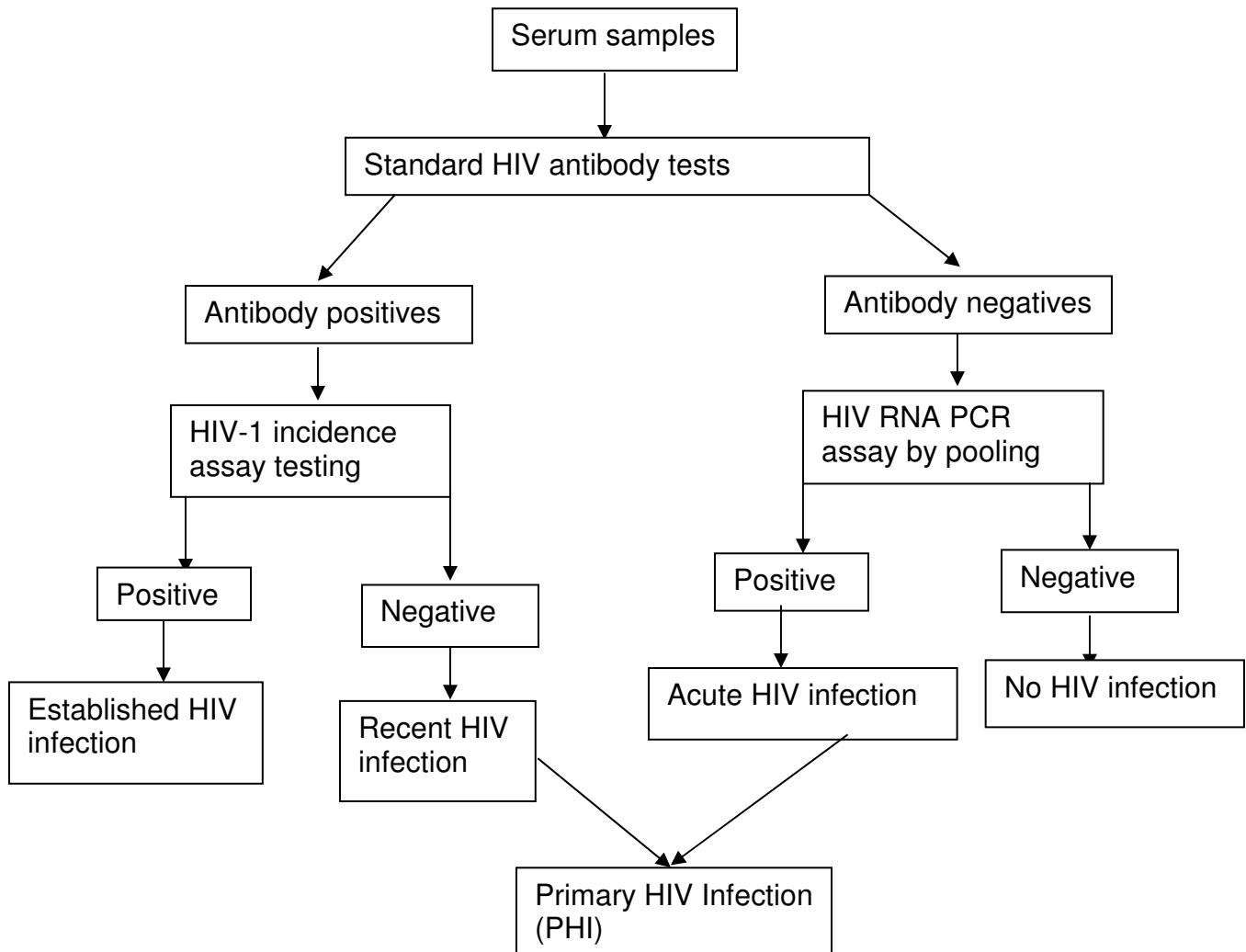
The IgG-capture HIV-1 BED incidence EIA (Calypte Biomedical Corporation, Maryland, USA) (31) was used to identify recent HIV-1 infections and estimate incidence. This assay uses a branched synthetic peptide that incorporates immunodominant gp41 sequences from divergent HIV-1 clades and detects increasing levels of anti-HIV IgG during early infection. The Calypte HIV-1 BED Incidence EIA is a quantitative antibody assay. Classification of individuals by this assay as recent seroconverters or long-term infections is based on average development of HIV-antibodies calculated from data using a large number of

people. However, there are differences among individuals and rates at which antibodies are produced. Although this assay is useful at the population level, its predictive value for the individual may be low and it is not recommended for individual diagnosis. The performance of the HIV-1 BED incidence EIA has been validated using a well characterized panel of serial serum specimens from 21 subtype C-infected seroconverters from an ongoing longitudinal cohort study in Ethiopia [Hailu et al, unpublished data] and Zimbabwe [unpublished data]. The validation study has been used to determine the cut-off Optical Density (OD) value to classify recent vs. long standing HIV-1 infection. Specimens that were initially positive by standard HIV antibody tests but negative on BED HIV-1 incidence assay were classified as recent HIV infections, while sera that were positive on both tests were classified as long standing/established HIV infections (30, 31, 32).

4.2.2 HIV RNA PCR Assay by Pooling

HIV antibody negative serum specimens were pooled in a 50:10:1: pyramid scheme and screened for HIV RNA using NucliSens assay (biomerieux) (13).

Fig. 1: Primary HIV Infection (PHI) Determination Flow Chart.



5. Data Analysis

Data was entered in to a computer using SPSS and analyzed using STATA statistical soft wares. Socio-demographic data was summarized by frequency tables and summary statistics. The incidence (rate of new infections/100 persons/year) was calculated using the following consensus formula: $I = [(365/W) \times (R)]/[N + (365/W \times R/2) \times 100]$, where I is incidence rate, W is mean window of detection, R is number of subjects found to be recently infected by the BED incidence assay and N is the total number of HIV-seronegative subjects (30). In earlier studies (1, 32), different formulae with minor variations have been used for calculating incidence. Recently, a consensus formula was agreed upon at the US CDC for calculating incidence. Calculated incidence does not differ significantly when different formulae are used. However, a consistent approach is recommended for rational comparisons among populations and trend analysis.

The 95% confidence interval (CI) for estimated BED incidence was calculated by the following formula: $95\% \text{ CI} = I \pm 1.96 (I/\text{Square-root of } R)$. Odds ratio with 95% confidence interval was used to measure the degree of association between risk factor and PHI. Logistic regression analysis was used to identify predictors of PHI.

6. Data Quality Control/Management

Serum samples were well labeled with waterproof marker and stored in a well-controlled -70 degree centigrade refrigerator at EHNRI lab. Specimen handling and testing was done at EHNRI laboratory with the advice and close follow up of an advisor who was well experienced in the field and with experienced

collaborating laboratory technicians. Standard Operating Procedures (SOPs) of each test was strictly followed. Every test in a series of laboratory testing was checked with its own controls. Each information was checked for its appropriateness and completeness before entering the data in to a computer and double entering of 10% of the data has controlled the quality of data.

7. Ethical Considerations

The initial project i.e. "Validation of the Syndromic Algorithm Approach for Management of Sexually Transmitted Infections (STIs) in Ethiopia"; was ethically approved by Research and Ethical Clearance Committee (RECC) of Ethiopian Health and Nutrition Research Institute (EHNRI) (33) as well as National Ethical Review Committee (NERC) of Ethiopian Science and Technology Commission's (ESTC) (34). In addition, at the time of the initial study, study subjects consented for their biological samples to be stored and be used in the future for any scientific research purposes. The analysis was anonymous and linked using unique identifiers; a condition in which ethical issue was not of much concern, but the Ethical Review and Clearance Committees of the Faculty of Medicine of Addis Ababa University and of EHNRI have approved the ethical aspects of the proposal in light of the above facts.

V. Results

1. Study Subjects

A total of 455 (321 female and 134 male) patients were recruited in the study.

Out of the total study subjects 392 (86.2 %) were from Addis Ababa and 63(13.8%) were from Southern Nations and Nationalities Peoples Region (SNNPR). Table 1 shows the distribution of socio-demographic characteristics of study subjects.

Table 1: Socio-demographic characteristics of study subjects, Ethiopia, 2006

Characteristic	Male; N = 134	Female; N = 321
Age Mean (range)	27.7 [15 – 49]	27.0 [15 – 49]
Median	26	25
Age Group: 15-19	22 (16.4%)	30 (9.3%)
20-24	33 (24.6%)	101 (31.5%)
25-29	33 (24.6%)	85 (26.5%)
30-34	18 (13.4%)	52 (16.2%)
35-39	11 (8.2%)	34 (10.6%)
40-44	9 (6.7%)	11 (3.4%)
45+	8 (6.0%)	8 (2.5%)
City/Site (HC): Addis Ababa	105 (78.4%)	287 (89.4%)
- Addis Ketema	15 (11.2%)	65 (20.2%)
- Arada	37 (27.6%)	83 (25.9%)
- Kazanchis	25 (18.7%)	46 (14.3%)
- Woreda 17	16 (11.9%)	54 (16.8%)
- Kirkos	12 (9.0%)	39 (12.1%)
SNNPR	29 (21.6%)	34 (10.6%)
- Awassa	16 (11.9%)	15 (4.7%)
- Bushelo	7 (5.2%)	13 (4.0%)
- Yirgalem	6 (4.5%)	6 (1.9%)

Characteristic	Male; N = 134	Female; N = 321
Education		
No schooling	23 (17.2%)	68 (21.2%)
Primary school	46 (34.3%)	122 (38.0%)
Secondary school	58 (43.3%)	126 (39.3%)
University	7 (5.2%)	5 (1.6%)
Religion		
Orthodox Christian	94 (70.1%)	244 (76.0%)
Muslim	18 (13.4%)	39 (12.1%)
Catholic	3 (2.2%)	1 (0.3%)
Protestant	19 (14.2%)	37 (11.5%)
Marital status		
Married	40 (29.9%)	140 (43.6%)
Single	86 (64.2%)	88 (27.4%)
Divorced	6 (4.5%)	51 (15.9%)
Widowed	2 (1.5%)	18 (5.6%)
Separated	0	24 (7.5%)
Others	0	2 (0.6%)

Regarding the study subjects' distribution by syndrome, 300 Vaginal Discharge Syndrome (VDS), 114 Urethral Discharge Syndrome (UDS) and 76 Genital ulcer Diseases syndrome (GUS) were complete for the required variables and lab results and entered in to analysis. Among the cases of VDS and UDS, 30 (10%) and 5(4.4%) were presented as double syndrome with GUS respectively. Thirty-five (46.1%) of the GUS cases were presented with additional genital discharges. In this case, the summation of the three syndromes is 490 as compared to 455 total study subjects and that is because of double counting of patients presented with double syndrome.

Table 2: Distribution of study subjects by syndromes, Ethiopia, 2006

Syndromes	Male No (%)	Female No (%)
Urethral discharge	109 (81.3%)	-
Vaginal discharge	-	270 (84.1%)
Genital ulcer	20 (14.9%)	21 (6.5%)
Vaginal discharge + Genital ulcer	-	30 (9.4%)
Urethral discharge + Genital ulcer	5 (3.7%)	-
Total	134 (100%)	321 (100%)

Regarding sociodemographic characteristics of patients identified to have PHI (22 patients), 72.7% of the cases were detected from Addis Ababa study participants and the rest 27.3% were from SNNPR who actually has contributed only 14% of the total study population. More than half (54.6%) of males lie in the age group 20 - 34 years and 36.4% lie above 35 years but all (100%) of the female cases lie in the age group 20 - 34 years. But considering both sexes together 15 of 22 PHI cases (68.2%) lie in the age group 20 – 29 years of age. More than half (54.5%) of males attended secondary school, whereas more than three-fourth (82%) of females attended either primary or secondary schools, the majority being secondary school (45.5%). About 64% of males were single but the same percent of females were married. More than a quarter (27.3%) of males were students and nearly half (45.5%) were government employees, merchants, daily laborers, farmers and unemployed in equal proportion. Whereas, nearly half (45.5%) of females were housewives and about 36.45% were unemployed.

2. Laboratory Tests

2.1 Standard HIV Antibody Serologic Tests

Of 455-stored serum samples 150(33%) had HIV infection as determined by standard HIV antibody tests, out of which 29 (19.3%) were males and 121 (80.7%) were females. HIV seroprevalence was 21.6% (29/134) among males and 37.7% (121/321) among females. With regards to the major STI syndromes, namely, VDS, UDS and GUS, HIV seropositivity was 35% (105/300), 20.2% (23/114) and 60.5% (46/76) respectively. In this case there is a possibility of a patient to be double counted when presenting with double syndrome. For example, if a patient presents with vaginal discharge and genital ulcer disease, this patient has a chance to be counted in vaginal discharge syndrome and also in genital ulcer disease syndrome during the analysis of HIV seropositivity with regards to the major STI syndromes. This condition could cause minor variations in the calculated and actual rates of HIV seropositivity.

HIV seropositivity was significantly associated with patient's presentation with double syndrome ($p < 0.001$). In the case of VDS, HIV seropositivity was 30.4% (82/270) as compared to 76.7% (23/30) when presented with VDS and GUS. In the case of GUS, HIV seropositivity was 46.3% (19/41) as compared to 68.6% (24/35) when presented with either VDS or UDS. But, no difference was noted in the case of UDS, as 20.2% (22/109) was found in UDS cases compared with 20% (1/5) in cases presented with UDS and GUS. Regarding the study sites, almost half of HIV positive cases were from Arada and Kazanchis health centers in Addis Ababa.

Table 3: Distribution of HIV seropositivity among the study patients by STI syndromes, Ethiopia, 2006

Syndrome	HIV Seropositive cases	
	Male: No (%)	Female: No (%)
UDS	22 (75.9%)	—
VDS	—	82 (67.8%)
GUS	6 (20.7%)	16 (13.2%)
VDS + GUS	—	23 (19.0%)
UDS + GUS	1 (3.4%)	—
Total	29 (100%)	121 (100%)

2.2 Primary HIV Infection (PHI) Determination

2.2.1 IgG-capture HIV-1 BED Incidence EIA

On the basis of the results of BED HIV-1 incidence assay, 15 (prevalence, 3.3%) out of 150 HIV antibody positive sera were identified as having recent HIV-1 infection and the rest 135 (prevalence, 29.7%) were identified as longstanding or established HIV infection. Out of the 15 recently infected cases 6 (40.0%) were males and the rest 9 (60.0%) were females. Prevalence of recent infections among the males was 4.5% (6/134) and 2.8% (9/321) among the females. Half of recently infected males were in the age group 35 - 49 years and attended secondary school, one-third were students and two-third were single. About 67% of recently infected females were in the age group 20 - 34 years and more than half (55.6%) were married and attended secondary school.

2.2.2 HIV RNA PCR Assay by Pooling

Of 305 HIV antibody negative serum samples, 15 were found to be either inadequate or were not of the required quality for PCR testing. Therefore, only

290 samples were tested by nucleic acid amplifications testing for HIV RNA by pooling method, but those 15 samples were considered as negative for HIV RNA during the analysis. Of 290 samples tested, 7 serum samples were identified as positive for HIV RNA, (prevalence, 1.5%). Out of the 7 acute HIV infection cases 5 (71.4%) were males and the rest 2 (28.6%) were females. Sixty percent of acute infections in males were in the age group 20 – 24 years, attended secondary school and single and, 80% of them were presented with UDS and were from a single place, Arada health center. All acute infections in the females were in the age group of 20 – 34 years, married and attended primary school. Half of them were housewives and the rest merchants.

3. Estimation of Incidence

In this study, annual HIV-1 incidence was estimated to be 11.8% (95% CI, (6.02, 17.58) on the basis of recent HIV-1 infections using the consensus formula. In a place where acute infections can be detected, annual HIV-1 incidence can be better estimated by considering both recent and acute infections. In this study considering both acute and recent infections and window period of 181 days (153 days for recent infection and 28 days for acute infection as determined by western studies) incidence rate was estimated to be 10.1% (95% CI, 5.13 - 14.97).

4. Risk Factors for PHI

In the analysis, 135 cases identified as having established or long-standing HIV infection of unknown duration were excluded and the remaining 320 cases taken

as denominator for the evaluation of risk factors. Out of 320, cases 22 were identified as having PHI, 15 recent and 7 acute infections.

Table 4: Univariate analysis of Risk factors associated with Primary HIV Infection (n=22) among patients presenting with conventional STIs, Ethiopian, 2006.

Characteristics	No. (%) of Subjects with PHI	Crude OR (95% CI)	P-value
Sex: Male	11 (50.0%)	1.00	0.12
Female	11 (50.0%)	1.98 (0.83, 4.72)	
Age group in yrs:			
15 - 24	7 (31.8%)	1.00	0.23
25 - 34	11 (50%)	1.82 (0.68, 4.84)	
35+	4 (18.2%)	1.52 (0.43, 5.42)	
Educational status:			
No schooling	5 (22.7%)	1.00	0.43
Primary school	5 (22.7%)	0.60 (0.17, 2.15)	
Secondary school & above	12 (54.6%)	1.16 (0.39, 3.44)	
Marital status:			
Single (never married)	9 (40.9%)	1.00	0.96
Divorced, widowed, separated	3 (13.6%)	0.96 (0.25, 3.71)	
Currently married	10 (46.5%)	1.11 (0.44, 2.83)	
Work involve overnight travel:			
No	17 (77.3%)	1.00	0.21
Yes	5 (22.7%)	1.95 (0.68, 5.60)	
Genital ulcer disease (confirmed on P/E):			
No	19 (86.4%)	1.00	0.63
Yes	3 (13.6%)	1.52 (0.34, 5.93)	
Regular sex partner: Yes	5 (22.7%)	1.00	0.75
No	17 (77.3%)	1.18 (0.43, 3.23)	
Genital discharge: No	2 (9.9%)	1.00	0.62
Yes	20 (90.1%)	0.68 (0.15, 3.13)	
Partner symptomatic:			
No	4 (18.2%)	1.00	0.08
Yes	3 (13.6%)	0.26 (0.69, 23.68)	
Don't know	5 (22.7%)	0.46 (0.36, 9.92)	0.32
No regular partner	10 (45.5%)	0.76 (0.22, 2.59)	0.66

Casual sex in the last 3 months:			
No	21 (95.5%)	1.00	
Yes	1 (4.5%)	0.31 (0.04, 2.35)	0.26
History of STIs:			
No	11 (50%)	1.00	
Yes	11 (50%)	1.42 (0.55, 3.66)	0.42
HSV2:			
NO	21 (95.5%)	1.00	
Yes	1 (4.5%)	0.74 (0.02, 5.18)	0.33
Syphilis:			
No	3 (13.6%)	1.00	
Yes	19 (86.4%)	6.56 (1.23, 31.67)	0.01*
Gonorrhea and Chlamydia			
No	17 (77.3%)	1.00	
Yes	5 (22.7%)	1.74 (0.61, 4.98)	0.29
Study area:			
SNNPR	6(27.3%)	1.00	
Addis Ababa	16 (73.7%)	0.44 (0.15, 1.33)	0.09

In univariate analysis (table 4), the only factor found to be significantly associated with PHI among the seronegative and recently HIV infected STI patients was diagnosis of syphilis (OR, 6.6; 95% CI, 1.57, 27.43; p=0.01). Also, in multivariate analysis, it was found to be independently associated with PHI, (AOR, 9.2; 95% CI, 1.56, 54.67; p=0.01). During the validation study HSV2 (69.7%) was the leading cause of GUS followed by syphilis (13.2%) and chancroid (3.9%). But it was syphilis found to be significantly associated with PHI. No association was found with other genital ulcers or genital discharge STI etiologies.

Analysis was also done separately for both sexes to identify predictors of PHI. Out of 134 male patients, 23 were identified as having established HIV infection and analysis was done on the remaining 111 patients. In male patients, age group 35 - 39 years was the only factor found to have significant association with PHI, (OR, 12.6; 95% CI, 1.07 - 148.13; p-value, 0.04) by univariate analysis but

was not independently associated by multivariate analysis. In females, out of 321 patients, 112 were confirmed to have established HIV infection and excluded from analysis, but no factor was found to have significant association with PHI.

VI. Discussion

HIV seroprevalence (33%) among Ethiopian patients presenting with STIs was high as compared to one study done in 1991, which was 12% among patients presented with all types of STIs (18) and lower as compared to another study done in 2001, which was 64% among patients presented with genital ulcer diseases (19). HIV prevalence in this segment of population was also very high as compared to the national HIV prevalence (4.4%) and also as compared to different routine and service related reporting sources which includes 4.5% among blood donors, 3.6% among VISA applicants and 16.6% among VCT clients (5). It was also found that HIV seropositivity was significantly associated with the presentation of patients with double syndrome ($p < 0.001$). Females were more affected than males with a prevalence of 37.7% and 21.6%, respectively. This condition could be attributable to diverse socioeconomic and cultural factors and this is an area that needs due consideration to empower women as a strategy to reduce the burden of HIV/AIDS in this segment of population.

The over all prevalence of recent HIV infection was found to be 3.3% (which is 10% of all HIV infection), and the percentage varies between the males and females, which was 4.5% and 2.8% respectively, but there was no statistically significant difference between the two, ($p = 0.118$). The national HIV prevalence

was 4.4%, which is both longstanding and recent infections, but the prevalence of recent infection alone (3.3%) in this study population was high indicating that epidemic is spreading in this population group. Further more, using statistical software package and based on ANC HIV surveillance data, the national HIV incidence was estimated to be 0.55% (5). In this study, based on recent infection data by BED HIV-1 incidence assay and using consensus formula, HIV-1 annual incidence was estimated to be 11.8%, which is much higher than the estimated national population-based incidence (0.55%) indicating that the epidemic is still spreading and more prevalent in this high-risk population group. This fact indicates that epidemic progression probability varies among different segments of population depending on their level of risk of exposure to the disease.

The prevalence of acute HIV infection among all study participants was 1.5% whereas 2.3% among the 305 seronegative participants. In this study, it was found that standard antibody tests detected only 95.5% of HIV infected individuals and the remaining 4.5% of infections were identified by nucleic acid amplification tests. Therefore, addition of such tests in high-risk populations such as patients presenting with STIs is justifiable and, even though it is expensive and technically demanding, it could be managed by collaborating with reference centers such as EHNRI. In one study done in Malawi, this fact has been demonstrated, indicating that more than two thirds of the acute HIV infection cases were detected in one sexually transmitted disease clinic, a clinic which has contributed only one third of the study participants (9). As nucleic acid amplification tests are expensive and technically demanding, PIHCT could be

strongly advocated as an alternative to these sensitive tests, so that patients could be put under strict follow up and be diagnosed early by the standard serologic tests for appropriate subsequent management of HIV infection in order to reduce the risk of HIV spread.

The current study showed that the high prevalence of acute HIV infection (1.5%), as compared to the national computer estimate of HIV-1 incidence (0.55%) in the general population, in this segment of population is a stronger justification than the fact that allows provision of post-exposure prophylaxis for HIV in cases of needle prick injuries during medical practice. The high viral load associated with acute HIV infection has critical public health importance since the magnitude of viral load is likely to predict the probability of sexual transmission of HIV. Recent studies of semen HIV dynamics indicated that viral loads in this compartment, while somewhat lower than those in blood, change in parallel with changes measured in blood (35).

Voluntary Counseling and Testing (VCT), nowadays, is at the center of HIV prevention and control activities. Currently, VCT centers in Ethiopia use standard serologic tests for HIV antibodies, which detect HIV infection of unknown duration or advanced diseases (Ethiopia, currently uses testing algorithm based on rapid tests as a standard test that were validated against ELISA and western blot). At that time, most sexual transmissions are likely to have occurred already (23). These tests do not differentiate recent from established or long-standing infections and also misses acute infections. Addition of incidence assays in these

centers would help to generate valuable data for incidence estimation in order to have a real picture of the dynamics of the epidemic in the country.

Detection of acute infections is important for the following reasons. First, it allows appropriate clinical management of a patient that might present with non-specific symptoms of acute retroviral infections, which also prevents inappropriate or unnecessary investigations for such patients. Secondly, it can help to prevent further transmission of the virus. The probability of transmission is very high during the first few months after acute HIV infection (23, 24) during which time patients have a high viral load in the blood and genital tract (36). Third, it can improve HIV surveillance. Standard antibody tests limit surveillance to the monitoring of populations living with latent or advanced HIV disease. In one study, the addition of nucleic acid amplification tests to the standard serologic antibody tests clearly demonstrated the potential of these tests to increase the accuracy and precision of data for both passive and active surveillance of HIV incidence (13).

A number of sociodemographic, clinical and behavioral factors were evaluated to identify associated factors with PHI and based on these factors to target individuals with increased likelihood of having PHI for subsequent and appropriate management of the infection. Diagnosis of syphilis (AOR, 9.2; 95% CI, 1.56, 54.67; $p=0.01$) was found to be an independent risk factor for PHI in patients presenting with conventional STIs. But, the current trend of STIs diagnosis and management in Ethiopia is syndromic approach and diagnosis of syphilis is difficult in a patient presenting with ulcerative STIs. Therefore, any STI

patient presenting with GUS with or without genital discharges could be considered as at high risk of having PHI.

Analysis was also done separately for both sexes to identify predictors of PHI. In male patients, age group 35 - 39 years was the only factor found to have significant association with PHI, (OR, 12.6; 95% CI, 1.07 - 148.13; p-value, 0.04) by univariate analysis but was not independently associated by multivariate analysis. In general these factors can be used to target patients when presenting with STIs for PIHCT at first contact in order that the risk of HIV transmission could be minimized during that critical period of HIV transmission. But the use of age group for males should be individualized and also it needs to be substantiated by further similar studies on multi-center and larger scale.

VII. Strengths and Limitations of the Study

1. Estimation of HIV-1 incidence on blood samples collected during cross-sectional survey by the current study is the first in its kind in Ethiopia and can be taken as one of the strengths of the study and, subsequently HIV-1 incidence can be estimated in different population subgroups to generate more accurate data for planning and resource allocation for effective preventive and control measures against HIV/AIDS.
2. The study was conducted on stored sera and other relevant data that were collected for other purpose, for validation study. The use of secondary data, coupled with primary data, which was generated by laboratory activities, could be an example of effective utilization of secondary data and this is one of the strengths of the study.

3. The study involved only two regions of the country, which was quite enough for the purpose of validation study, and that may not be representative of the nation particularly regarding socio-economic and cultural factors for identification of associated factors with PHI that can be used at national level and that was considered as a main limitation of the study.

VIII. Conclusions and Recommendations

The current study, which is the first in its kind in Ethiopia, has enabled us to estimate the incidence of HIV infection in patients presenting with conventional STIs (one of known risk groups for HIV infection). High incidence (10.1% per year) of HIV infection in this group necessitates targeted interventions as part of the fight against HIV/AIDS epidemic in the country. Therefore we recommend that one of the targeted interventions should be strong and sustained encouragement of STI patients for PIHCT, so that individuals would be managed appropriately and the risk of transmission of the infection to the public would be remarkably reduced.

Population based estimation of incidence of HIV infection can be made to increase the accuracy and precision of data through both passive and active surveillance of HIV/AIDS in the country to assist in prevention programs, targeting resources and monitoring and evaluation of effectiveness of prevention and control programs in fighting the epidemic. Recently, the HIV-1 BED Incidence EIA has been used in a number of cross-sectional population studies to estimate incidence and evaluate association with various risk factors (37) such as the current study. Therefore, similar types of studies could be scaled up on different population subgroups cross-sectionally, so that a clear picture of the dynamics of the epidemic could be brought on board in Ethiopia.

The prevalence of acute HIV infection (1.5%) was also high in this study population. Therefore, post exposure prophylaxis for HIV in this population group,

especially those presenting with genital ulcer diseases, is the issue to be considered though it could be a debatable issue and needs policy decision. But we feel that high prevalence of acute HIV infection in this subgroup of population is a stronger justification than the fact that allows provision of post-exposure prophylaxis in cases of needle prick injuries during medical practice despite the stronger ethical indication for the later group and, we recommend that MOH should seriously consider this issue for its realization.

Diagnosis of syphilis (or genital ulcer diseases, since the trend in Ethiopia is syndromic approach) was identified as an independent predictor of PHI for both sexes and one can use this factor to identify patients at high risk of having PHI when presenting with conventional STIs, particularly genital ulcer diseases for subsequent and strong counseling for PIHCT but should be individualized for patients presenting with genital discharges. In addition, age group 35 – 39 years for males was found to be significantly associated with PHI. Therefore, this factor can be used to identify male patients at high risk of having PHI when presenting with conventional STIs for subsequent strong and sustained PIHCT and it is also recommended that further similar studies be conducted to substantiate these findings.

IX. Dissemination Plan

Results from this study, after being defended for a partial fulfillment of MPH, will be communicated to relevant and interested bodies and will also be presented at national, regional or international meetings. Finally it will be submitted for publication to national or international peer-reviewed journals, as deemed necessary and/or found suitable.

X. References

1. Janssen RS; Satten GA; Stramer SL, et al. **New Testing Strategy to Detect Early HIV-1 Infection for Use in Incidence Estimates and for Clinical and Prevention Purposes.** *JAMA* 1998; 280(1): 42-48.
2. McFarland W, Busch MP, Kellogg TA et al. **Detection of early HIV infection and estimation of incidence using sensitive /less sensitive enzyme immunoassay testing strategy at anonymous counseling and testing sites in San Fransisco.** *J Acquir immune Defic Syndr* 1999 Dec 15; 22(5): 484-9
3. Schwarcz S, KelloggT, McFarland W, et al. **Differences in the temporal trends of HIV seroincidence and seroprevalence among sexually transmitted disease clinic patients, 1989-1998: application of the serological resting algorithm for recent HIV seroconversion.** *AM J Epidemiol.* 2001; 153(10): 925-34
4. UNAIDS/WHO. **AIDS epidemic update; special report on HIV prevention.** Geneva, Switzerland; December 2005.
5. MOH, **AIDS in Ethiopia: 5th edition,** June 2004
6. WHO/HIV-AIDS/2001.02. **Estimated new cases of curable STIs among adults, 1999.**
7. UNAIDS **Questions and Answers,** August 2004
8. Morandi, PA et al. **Detection of human immunodeficiency virus type 1 (HIV-1) RNA in pools of sera negative for antibodies to HIV-1 and HIV-2.** *J. Clin. Microbiol.* 1998. 36(6): 1534-1538.
9. Pilcher, C.D et al. **Frequent detection of acute primary HIV infection in men in Malawi.** *AIDS* 2004. 18(3):517 - 524.
10. Pilcher, CD. et al. **Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population.** *JAMA* 2002. 288(2): 216-221.
11. Cameron, DW et al. **Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men.** *Lancet* 1989; 334(8660): 403-407.

12. Bollinger RC, Brookmeyer RS, Mehendale SM, Paranjape RS, Shepherd ME, Gadkari DA, *et al.* **Risk factors and clinical presentation of acute primary HIV infection in India.** *JAMA* 1997, 278(23): 2085-2089.
13. Pilcher CD, *et al.* **Detection of Acute Infections during HIV Testing in North Carolina.** *N Engl J Med* 2005; 352(18): 1873 -1883
14. Desta S, Feleke W, Yosuf M. **Prevalence of STD and STD related factors in sex workers of Addis Ababa.** *Ethiop J Health Dev.* 1990; 4(2): 149-53
15. Gameda A. **Magnitude of STDs problem in Ethiopia.** Ministry of Health, 1990.
16. Geyid A, Tessfaye H, Abraham A, Lemeneh Y, Desta S, Feleke W. **Isolates of STD causative agents from sex workers in Addis Ababa (a preliminary report).** *Ethiop J health Dev* 1990; 4(2): 155-61
17. Girma A, Geyid A, Mekonnen Z, *et al.* **Validation of the Syndromic Algorithm Approach for Management of Sexually Transmitted Infections (STIs) in Ethiopia.** EHNRI-CDC collaborative research (Unpublished data), 2003.
18. Kefeni H, Desta B, Mengesha S, Zewdie D, Kebede T. **Prevalence of HIV-1 antibodies in patients with STDs.** *Ethiop Med J* 1991; 29(2):63-69
19. Wolday D, Mariam Z, Mohammed Z, *et al.* **Risk factors associated with failure of syndromic management of sexually transmitted diseases among women.** *Sex Trans Infect* 2004; 80(5): 392-394.
20. Mihret W, Rinke D Wit TF, Petros B, *et al.* **Herpes simplex virus type 2 seropositivity among urban adults in Africa: results from two cross-sectional surveys in Addis Ababa, Ethiopia.** *Sex Transm Dis* 2002; 29(3): 175-181.
21. Wolday D, Mariam Z, Mohammed Z, *et al.* **Prevalence of herpes simplex type 2 virus among patients presenting with STDs compared to the general community in Addis Ababa.** 40th Ethiopian Medical Association Annual Scientific Conference, June 2004.

22. Wolday D, Mariam Z, Mohammed Z, et al. **The impact of Syndromic treatment of sexually transmitted diseases on cervical shedding of HIV-1.** *AIDS* 2004; 18(5): 781-85.
23. Wawer, M.J., et al. 2003. **HIV-1 transmission per coital act, by stage of HIV infection in the index partner, in discordant couples, Rakai, Uganda.** Program and abstracts: 10th Conference on Retroviruses and Opportunistic Infections. Boston, Massachusetts, USA. Abstr. 40.
24. Pilcher CD, Joseph J. Eron, Jr., Shannon Galvin, Cynthia Gay and Myron S. Cohen. **Acute HIV revisited: new opportunities for treatment and prevention** *J. Clin. Invest* 2004; 113(7): 937-945.
25. Dyer, J.R. et al. **Shedding of HIV-1 in semen during primary infection.** *AIDS* 1997; 11(4): 543-545.
26. Chakraborty, H. et al. **Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: a probabilistic empiric model.** *AIDS* 2001; 15(5): 621-627.
27. Fiebig EW, Wright DJ, Rawal BD, et al. **Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection.** *AIDS* 2003; 17(13): 1871-1879.
28. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. **The risk of transfusion-transmitted viral infections: the Retrovirus Epidemiology Donor Study.** *N Engl J Med* 1996; 334(26): 1685-1690.
29. Daar ES, Little S, Pitt J, et al. **Diagnosis of primary HIV-1 infection. Los Angeles country primary HIV infection recruitment network.** *Ann Intern Med* 2001; 134(1): 25-29.
30. Parekh, Bharat S, McDougal, J steven. **Application of laboratory methods for estimation of HIV-1 incidence.** *Indian J Med Res* 2005; 12(4): 510-518
31. Dobbs T, Kennedy S, Pau C-P, McDougal JS, Parekh BS. **Performance characteristics of the immunoglobulin G-capture BED-enzyme immunoassay, an assay to detect recent human immunodeficiency virus type 1 seroconversion.** *J Clin Microbiol* 2004; 42(6): 2623-2628.

32. Parekh BS, Kennedy MS, Dobbs T, et al. **Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence.** *AIDS Research & Human Retroviruses* 2002; 18(4): 295-307
33. Research and Ethical Clearance Committee (RECC) of Ethiopian Health and Nutrition Research Institute (EHNRI. July 24, 2001: 24th minutes
34. National Ethical Review Committee (NERC) of Ethiopian Science and Technology Commission (ESTC). 4 Feb 2002: Ref. No. RDHE/60 - 42/2002.
35. Pilcher CD, Shugars D, Fiscus SA, et al. **HIV in body fluids during primary HIV infection: Implications for pathogenesis, treatment and public health.** *AIDS* 2001, 15(7): 249-258
36. Pilcher CD, Tien HC, Eron JJ Jr, et al. **Brief but efficient: acute HIV infection and the sexual transmission of HIV.** *J Infect Dis* 2004; 189(10): 1785-1792.
37. Hu D, Vanichseni S, Mock PA, et al. **HIV-1 incidence estimates by detection of recent infection from a cross-sectional sampling of injection drug users in Bangkok: Use of the IgG Capture BED enzyme immunoassay.** *AIDS research and Human Retroviruses.* 2003, 19(9): 727-730.

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Declaration

I the undersigned, declare that this is my original work, has never been presented in this or any other university and that all the source materials used for the thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as a university advisor.

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