EVALUATION OF SOME FACTORS INVOLVED IN HETEROSEXUAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS AMONGST SPOUSES SEEN AT KENYATTA NATIONAL HOSPITAL (KNH)

BY

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DECLARATION:

This dissertation is my original work and has not been presented in any University.

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LIST OF ABBRÈVIATIONS:

HIV -1, 2.....Human Immunodeficiency Virus type-1 and type-2 respectively.

AIDS.....Acquired Immunodeficiency Syndrome.

ARC.....AIDS Related Complex.

HTLV-I,II,III..Human T-cell lymphotropic Virus types I, II and III respectively.

T_4(CD4+).....T-helper/inducer lymphocytes

T_8(CD8+)..... T-suppressor lymphocytes

WHO..... World Health Organization

STDs..... Sexually Transmitted Diseases

HLA..... Human Leukocyte antigen

S.D..... Standard Deviation

SPSS ..... Statistical package for Social Sciences

GUDVD..... Genital Ulcer Disease and other Venereal Diseases.

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A - SUMMARY

Between February and October 1993 (inclusive) 120 HIV-infected persons (index cases) were enrolled into the study at the Kenyatta National Hospital to determine behaviours associated with acquisition of HIV-infection and the risk factors associated with transmission of the infection to their 123 spouses (sexual partners).

The male to female ratio in the index cases was 1.3:1 and for the sexual partners the ratio was 1:1.4. Of the 123 sexual partners 87 (70.7%) were HIV-positive and 36 (29.3%) were seronegative. None of the seronegative partners who had repeated testing seroconverted in the six months of follow up.

Female index cases were younger [mean age 28.4 (SD=6.9) years] than the males [mean 36.1 (SD=8.2) years] (t-test; $p<0.001$). Of the 51 male spouses 33 (64.7%) were HIV-Positive and of the 72 females 54 (75%) were HIV-Positive but the difference is however not significant ($p > 0.05$).

History of multiple other sexual partners (besides index cases) in association with other STDs were found to be associated with HIV-infection among spouses (especially females). Though there was a high prevalence of multiple injections at various health facilities among spouses, there was no significant difference between the HIV-positive and seronegative sexual partners in the study and the

protective role of condoms was not fully evaluated. Blood transfusion and intravenous drug use were not found to be risk factors for HIV-infection of spouses in the study.

Progressive depletion of CD4+ T-lymphocytes (helper cells) in HIV-positive individuals was observed in the study. Index cases had a significantly lower mean CD4+ count of 143 cells/ml (SD=243) compared with the sexual partners with a mean CD4+ count of 523 cells/ml (S.D = 281) (t-test; $p<0.001$). In the same trend, the HIV-positive spouses had a significant lower mean CD4+ count of 432 cells/ml (S.D. = 237) compared with HIV-negative spouses with a mean count of 711 cells/ml (S.D.=233) (t-test; $p<0.001$). It was also found suggestive in the study that the lower the CD4+ count of the index cases, the higher the transmission rate of the HIV-infection to their spouses.

Anaemia resulting from various factors associated with HIV-infection was noted in the study. Index cases had a mean haemoglobin of 11.1g/dl (S.D.=2.4), HIV-positive spouses of 13.2 g/dl (S.D. = 1.9) and seronegative spouses of 14.6 g/dl (S.D. = 1.7).

There was no association between the clinical stage of index cases with the HIV-serological status of their spouses. The outcome of HIV-infection was generally found to be poor. Sixty two percent of index cases had AIDS (stage 4 disease) at recruitment and by the

end of the 6 months follow up 54.7% of them were dead. A drop out rate of 44.7% of the spouses was observed during the study.

B. INTRODUCTION/LITERATURE REVIEW

General Aspects of HIV

Human immunodeficiency virus type 1 (HIV-1) has been clearly identified as the primary cause of acquired immunodeficiency syndrome (AIDS) (1). Unlike other infectious diseases in the late twentieth century, AIDS is currently a constant pre-occupation of scientists, physicians and the general public world wide.

Despite extensive research in the field of AIDS since the last decade (1980's), there is as yet no effective specific treatment for AIDS (2). The development of an effective vaccine for HIV-1 is hindered by several obstacles such as:

(i) the failure to delineate the viral components or epitopes that induce protective immunity in the host;

(ii) the definition of protective immunity against initial infection with HIV-1 remains unknown;

iii) the delineation of effective immunity against development of disease in already infected persons is not well understood (3).

This leaves modification of risk factors for HIV-transmission as reliable tools at our disposal to interrupt the transmission of HIV-1 infection as of today. It is for this reason that epidemiological studies still form the cornerstone in the management of the AIDS pandemic.

The Agent

Historical background

The acquired immunodeficiency syndrome (AIDS) was first reported in the United States in 1981, in previously healthy male homosexuals who presented with opportunistic infections and Kaposis sarcoma (3). The term AIDS was officially adopted in 1982. The causative retrovirus called lymphadenopathy associated virus (LAV) was identified first by Montagnier and colleagues in Paris (4). Simultaneously, Gallo et al reported isolation from patients of a virus which they called Human T-cell lymphotropic virus type 111 (HTLV-111) (5). Investigations confirmed the similarity of LAV and HTLV-111 and by international agreement the virus is now referred to as Human immunodeficiency virus type 1 (HIV-1). The most recently described retrovirus is Human Immunodeficiency Virus type-2 (HIV-2). It has 40% similarity to HIV-1 (6). This has been described in West Africa, and is relatively an uncommon and inefficient cause of AIDS. Its spread is not with as much rapidity as HIV-1 (7).

Life cycle and Immunopathogenesis of HIV-1 Infection

(i) Abnormalities of cellular immunity:

Helper T-lymphocytes that express CD4+ (T4) cell surface molecule play a key role in the human immunodeficiency virus infection. The CD4+ molecule found in lower concentrations on monocytes, macrophages, dendritic and glial cells and certain endothelial cells in the intestine serve as high affinity receptors for HIV-1 (8,9).

The viral envelope protein gp120 binds to a region of CD4+ resulting in internalization and uncoating of the virion. The viral genome is then transcribed by reverse transcriptase to proviral DNA that is subsequently incorporated into host DNA. The virus may then remain latent for variable periods of time and may be reactivated to replication when the host T-cell is stimulated by other immunologic factors. Other viral infections like cytomegalovirus, Herpes Simplex and Epstein Barr virus together with some cytokines like tumour necrosis factor - alpha (TNF- α) are some of the external stimuli thought to reactivate viral replication in the T4-lymphocytes (Helper/Inducer cells) (9).

Once the virus is activated, its proviral DNA is Transcribed into messenger RNA ultimately leading to protein synthesis, viral assembly and release of viral particles (10).

Several mechanisms of CD4+ (T4) cell death have been proposed including cytolysis as a result of active viral replication and development of syncytia (aggregation of cells). As the new virions bud from the plasma membrane, the host lymphocytes fuse with the surrounding uninfected CD4+ cells creating multinucleated giant cells leading to death of both infected and uninfected cells (10). The net result is a gradual and progressive diminution of CD4+, T-lymphocyte numbers which occurs in almost all HIV-infected persons. This ultimately leads to serious immunodepression and subsequent

opportunistic infections characteristic of acquired immunodeficiency syndrome (AIDS) (10).

It has now been observed that the lower the T4-lymphocyte counts of an HIV-infected individual, the higher the transmission rate of the virus to uninfected heterosexual partner during coitus (11).

(ii) Abnormalities of humoral immunity

One of the earliest immunologic manifestations of AIDS is a polyclonal hypergammaglobulinemia which is possibly related to infection by viruses such as Epstein Barr virus (EBV), cytomegalovirus (CMV) or HIV- itself (8). HIV-antibodies in the serum of infected individuals, are detectable approximately between 4 to 12 weeks after infection (12,13).

In a study carried out by Fischl et al (14) amongst 45 heterosexual partners of adults with AIDS, 29% had antibody to HTLV-III at enrollment. Of the 32 spouses who were seronegative at enrollment, 13 (41%) developed antibody to HTLV-III during the 18 month course of the study.

Apart from the consequences of the diminishing CD4+ T-helper cells in initiating specific antibody production, intrinsic B-cell physiology is abnormal in HIV-Seropositive individuals. The B-lymphocytes from the patients are perpetually in a state of polyclonal activation. In affected adults, serum levels of IgG,

IgA and IgD are increased but 1gM levels are relatively normal. In sharp contrast to the spontaneous hyperactivity, antigen-specific and non-specific B-cell responses are impaired in AIDS patients. It has then been noted that AIDS patients have poor responses to primary and secondary immunizations with protein and polysaccharide antigens and are prone to pyogenic infections (12,13).

Incubation Period and clinical manifestation of HIV-infection

The estimated mean period from time of infection to the development of clinical AIDS is observed to be more than 5 years (15). The clinical spectrum of HIV-infection ranges from an asymptomatic stage to a full blown AIDS picture as has been described by the Centres for Disease Control in the U.S.A. (16,17).

The clinical features might be as a direct consequence of HIV or due to the opportunistic infections or tumours occurring as a result of immunodepression. Virtually every system of the body is affected in the clinicopathological spectrum of AIDS. There are now established manifestations of AIDS in virtually all tissues and organs (18-25).

In the study carried out by Fischl et al (14) amongst spouses of 45 adults with AIDS, 42% heterosexual partners developed clinical disease in the 12 months of follow up. Nine of the spouses had lymphadenopathy alone, 6 developed an AIDS related illness and 4 developed AIDS (CDC-classification).

In another study conducted by Marowa et al (26) in Zimbabwe amongst 75 HIV-infected men, 5.3% of them were asymptomatic, 53.3% had persistent generalised lymphadenopathy (PGL), 32% had AIDS related complex (ARC) and 9.4% had AIDS. HIV-serological status of women married to the men was compared with the clinical stage of HIV-infection of their husbands. It was observed that there was greater probability that a wife became seropositive if the husband had ARC or AIDS (26).

Epidemiology of Heterosexual transmission of HIV-1

The epidemiology of heterosexual transmission of HIV-1 falls in WHO Pattern II areas (27,28). These include Sub-Saharan Africa, countries in the Caribean and Latin america. The male to female ratio of HIV-infected and AIDS cases is approximately equal (1.1:1) in these areas.

To-date the WHO estimates a worldwide total exceeding 13 million HIV-infected adults (29). About 80% of the cases are from developing countries and it is thought that two-thirds have occurred in Sub-Saharan Africa. However, HIV-incidence rates due to heterosexual transmission have been rapidly increasing in South East Asia, Eastern Europe and even in the Americas (29).

TRANSMISSION OF HIV-1

When AIDS was first reported in the United States it was shown by Gottlieb and others that the majority of those infected could be delineated into certain characteristic groups such as: homosexual or bisexual men, intravenous drug users, blood transfusion recipients, haemophiliacs and infants born to mothers with HIV-infection (30-32).

Homosexual activity and intravenous drug use

Various reports have confirmed that numerous homosexual partners and frequent receptive anal intercourse accounts for a high prevalence of HIV-infection among male homosexuals in the United States and Western Europe (33, 34). On the other hand little work has been done on lesbians. Two case reports in literature suggested intimate sexual contact between women, may be able to transmit HIV. In both cases oral exposure to vaginal fluid or menses was suggested as the possible mode of transmission and in one case the partners had vaginal bleeding as a result of traumatic sexual activities (35,36).

Among drug users, the risk of being infected with HIV is closely linked to both the frequency of drug injection and the sharing of needles or injection with contaminated needles. These factors are analogous to frequent receptive anal intercourse and numerous homosexual partners (37). Homosexuality and intravenous drug use however have not been found to be risk factors for HIV-transmission

in Africa.

Heterosexual Transmission of HIV-1

(a) General aspects of heterosexual transmission:

HIV has been isolated from semen and cervical secretions and it appears to be efficiently transmitted to either partner by heterosexual penile-vaginal intercourse (38,39).

Risk factor analysis has shown that seropositivity is related to the number of heterosexual partners, to prostitution and/or to heterosexual partners having AIDS or HIV-antibodies. Harris and colleagues demonstrated immunodeficiency in female sexual partners of men with AIDS (40). This was further confirmed by studies done on partners of haemophiliacs (usually males) that the only risk factor to the females were the infected male spouses (41). Male to female heterosexual transmission of HIV-1 infection was then proven. Female to male transmission was also proven by various studies like that of Van-de-Perrep et al which looked at female prostitutes as a risk for HIV transmission (42).

In Sub-saharan Africa heterosexual transmission of HIV as a predominant risk factor has been demonstrated. A hospital based study in Zambia by Melbye et al found that 17.5% of the studied group had HIV antibodies with the highest prevalence in sexually active groups of men aged 30 to 35 years and women aged 20 to 25 years (43).

Another study done in Zaire showed very high HIV infection rates in a heterosexual population including 6% hospital workers and 31% of female prostitutes in Kinshasa (44). The prevalence of HIV antibodies in a study done among non-drug using prostitutes in Pumwani, Nairobi was 31% to 61%. Among the seronegative prostitutes, the 2-year HIV-seroconversion rate was 56% suggesting an alarming transmission rate (45).

Risk factors for heterosexual transmission for HIV

Risk factors for heterosexual transmission of HIV-1 include: history of other sexually transmitted diseases (STDs), genital ulcer disease, use of oral contraceptives, degree of immunodepression, lack of male circumcision and some genetic factors.

1. History of other sexually transmitted diseases (STDs) and/or genital ulcer disease (GUD):

Epidemiologic studies carried out during the early years of the HIV epidemic disclosed that a past history of sexually transmitted diseases was obtained from patients with AIDS. The STD clinics, were used for HIV-screening and it was found that clinic attenders, had a higher prevalence of HIV-infection than the overall population (46). For example in 1983 a study of 90 homosexual men with generalised lymphadenopathy, all had a past history of STD; 83% with past episode of gonorrhoea; 44% with condyloma acuminata, 24% with syphilis and 19% with genital herpes (47).

Numerous studies have shown that STDs are extremely prevalent in

Sub-Saharan Africa and that they share with HIV, the same risk behaviours of multiple sexual partners, same route of transmission and the same human reservoir, sexually active adults (47,48).

Hypotheses have been proposed to explain the discrepancy in the apparent ease of heterosexual transmission of HIV in Africa compared with that of the western countries. Behavioural factors have been implicated; prostitution is thought to be more readily available in Central Africa (47,49). However the HIV epidemic has spread beyond the 'core' prostitutes and their clients. It is thought the cofactors most likely to be implicated in the African HIV epidemic are the classic sexually transmitted diseases (STDs). The STDS could act directly by increasing infectivity of the seropositive individuals or by enhancing the susceptibility of their contacts or both (48,49).

Genital ulcer disease (GUD) has been identified as an independent risk factor for HIV-seroconversion in a cohort of prospectively followed prostitutes in Nairobi (50). Among men with a recent history of sexual intercourse with a prostitute seen at an STD clinic, HIV-seronegative men presenting with genital ulcer disease were more likely to seroconvert than were men presenting with urethritis (50, 51). STDs therefore could be the major cofactors for HIV-transmission. Inadequate management of STDs in terms of diagnosis and treatment which is a likelihood in Africa due to inadequate medical facilities, could enhance and/or accelerate

heterosexual transmission of HIV in this region.

2. **Oral contraceptives as cofactors for heterosexual transmission of HIV:**

In a prospective study of Nairobi prostitutes, the incidence of HIV infection was significantly greater in oral contraceptive users than non-users (52). Though the oral contraceptive protects from pregnancy, its encouragement of promiscuity predisposes the individual to HIV-infection. However, oral contraceptives seem to have an independent association with HIV-transmission besides the promiscuity practiced by the users. The association between oral contraceptive use with HIV-infection persisted after controlling for such covariants as number of sexual partners, STDs and condom use. The association with the oral contraceptive use though not yet confirmed by other studies may be due to general mechanisms including:-

(i) alterations in cell-mediated immunity with changes in the distribution of target cells in the female genital tract,

(ii) increased cervical ectopy and

iii) enhanced transmission of other sexually transmitted *diseases (53,54).*

3. **Degree of immunosuppression as cofactor for heterosexual transmission of HIV-1:**

A third cofactor for sexual transmission of HIV may be the degree of virus expression and/or immunosuppression in the seropositive "donor". In a study of 24 wives of seropositive haemophiliacs, four women developed HIV-antibodies. The husbands of the women who

became infected tended to have lower absolute T_4 lymphocyte count than the husbands of the women who remained uninfected (55).

The results were consistent with those of a study of congenital transmission in Zaire which found that the risk of transplacental infection to the fetus was inversely related to T-helper cell levels in the mother (56). These studies both suggest that the seropositive individual's infectivity increases with the duration of infection and severity of disease. It is possibly thought that HIV-related immunosuppression is associated with increased plasma viremia (57). This also suggests that more heterosexual transmission will occur as more heterosexuals enter this more infectious stage of HIV-infection.

4. <u>Role of foreskin (lack of circumcision)</u>:

Increased risk of acquisition of HIV-1 in uncircumcised men has been consistently demonstrated in case-controlled cohort studies (51, 58). Anthropological data based on ethnic group practice of male circumcision and population based HIV-1 seroprevalence data support the hypothesis that uncircumcised men are at much greater risk of HIV infection (51, 59).

The mechanisms by which the foreskin increases susceptibility to HIV-1 are unclear. However, chancroid and other genital-ulcer diseases develop more commonly in uncircumcised men. It is also thought that perhaps the occurrence of balanitis, maceration of the

lans penis, or trauma of the intact prepuce during intercourse ccounts for the association of HIV-prevalence with the presence of foreskin (51). In addition the microenvironment under the oreskin could permit longer survival of HIV on the epithelium and ius more time for penetration.

ie relation of HIV infection to the presence of a foreskin also iggests that the most frequent portal of entry of HIV in the male enital tract, is the glans penis and/or penile shaft rather than he urethra since the exposure of urethral mucosa to HIV-infected ecretions during heterosexual intercourse is presumably no less in ircumcised men than in uncircumcised (51). This issue however eeds further evaluation in other populations.

. <u>The role of genetic factors</u>:

inally it is possible that genetic factors may influence usceptibility to HIV. A study in England found that various llelic forms of group specific component were associated with both IV-infection and disease progression (60). Different epitopes on D4+ molecule may also determine susceptibility to HIV-infection, ut available results need confirmation (61).

BO blood grouping and HLA- tissue typing are currently being nvestigated as possible markers for either HIV susceptibility r protectivity. A study conducted among prostitutes in Nairobi evealed that some HLA-markers tended to be protective against HIV-

infection. This include A28 and B27 (in class I) and DR 13 (Class II) alleles all being associated with decreased seroconversion for HIV-antibodies (Plummer F., Personal Communication).

The role of condoms in prevention of heterosexual transmission of HIV:

With the advent of other methods of contraception and cures for venereal infection, interest in the use of condoms had drastically declined worldwide until the fear of AIDs influenced their recent much publicized resurgence. Rosernberg reported back in 1987 that the actual capacity production of condoms worldwide was unknown, but in the United States it was estimated that 5 billion devices were produced annually (62). The figure is undoubtedly higher currently.

According to surveys carried out in 1982 by Sherris and colleagues, (63), it was noted that condoms were most used in Japan (among 50% spouses of all married women of reproductive age) and least in Africa (less than 1% of spouses of such women). In developed countries overall the rate of condom use was about 13% (63). In Africa, particularly, various myths surround the use of condoms. Most of them based on unreliable information, could explain the least use of condoms by males.

The admonitions to employ condoms so as to enjoy "safer sex" are based on more than wishful thinking. Several studies have demonstrated the impermeability of condoms to sexually transmitted

diseases including HIV. For example Conant and colleagues conducted studies to prove that condoms are effective barriers against transmission of herpes virus and human immunodeficiency virus (64). In the study he tested 12 varieties of latex condoms against transmission of herpesvirus 1 and 2, cytomegalovirus and HIV. None of the agents passed through latex while there was some leakage with the natural condom (62,64-65).

Currently most condom manufacturing and importing countries have national standards for quality control. This takes into account the texture, sizes, style of application and sales. Latex condoms are safer than natural - membrane condoms made of lamb ceacum (65).

A study done by Ngugi and colleagues among prostitutes in Nairobi after a programme of AIDS and STDs education plus free distribution of condoms found a striking increase in condom use. The study also found that any condom use resulted in a 3-fold reduction in risk of seroconversion for HIV-antibodies (66).

The high efficacy of condoms in preventing HIV-infection is thought to reflect a multiplier effect in condom use. That is a part from reducing the risk of exposure to HIV, condoms also decrease the susceptibility to HIV by preventing other STDs that may facilitate acquisition of HIV, such as genital ulcer disease as has been previously discussed (66).

previously discussed (66).

Failure rate of condoms:

Concerning the "failure rate" of condoms used for contraception, 10% is an often cited figure (65). Conant, however argued that no study has ever been carried out. His clinical impression was that non-compliance with improper recommendations for condom use, rather than product failure is responsible (65). Hatcher's "condom sense" rules are now recommended to be strictly adhered to for proper condom use especially in the prevention of STDs including HIV (67).

OTHER MODES OF TRANSMISSION OF HIV-1

Blood transfusion, organ transplant and other related therapies:

The first report of AIDS in haemophiliacs provided evidence that HIV was not only transmitted by intimate sexual contact but also by blood and plasma products. Studies have confirmed that the likelihood of becoming infected is related to the dose of commercial factor VIII concentrate administered (68).

HIV has been transmitted from HIV - infected donors by kidney transplantation and artificial insemination; and by extension any other donated organ or tissue are potential risk factors for HIV-transmission (69).

Exposure to Needles, and other Body Fluids:

Studies of a large group of nurses and other health care workers with intensive exposure to AIDS have shown HIV-seroconversion only as a consequence of parenteral injuries with contaminated needles or other sharp instruments (70, 71). With regard to nonparenteral exposure of blood, feces, urine and other body fluids as a mode of HIV-transmission individual cases have been documented by anecdotal reports (72, 73).

Repeated therapeutic injections appear to be related to HIV-seropositivity among children who had HIV-negative mothers in Kinshasa, Zaire (74). This work however has not been reproduced elsewhere.

C - AIMS AND OBJECTIVES

1. To determine the proportion of spouses of HIV-infected persons (index cases) that are seropositive/seronegative for HIV-antibodies.

2. To determine other possible risk factors besides the HIV-positive index cases in the HIV-positive spouses such as:-

(i) Other sexual partners

(ii) Blood transfusion

(iii) Genital ulcer disease and/or other venereal diseases

(iv) Intravenous drug use

(v) Multiple injections at various health facilities.

3. To determine possible protective factors in the HIV-negative spouses such as:-

(i) Use of condoms

(ii) ABO-blood group

(iii) Male circumcision

4. To assess the clinical health status of the HIV-infected index cases and their spouses during the study.

5. To determine some haematological and immunological parameters (Haemogram, T-lymphocytes-CD4+ and CD8+ subsets) of the HIV-infected index cases and their spouses.

D - JUSTIFICATION FOR THE STUDY

Though various studies expound the concept of heterosexual contact as a route of transmission of HIV-1, the efficiency, risk factors and/or mechanisms associated with heterosexual transmission of HIV-1 are not yet fully understood.

Several important questions related to HIV-1 heterosexual transmission remain to be answered for example:

(a) how could one explain the fact that a certain cohort of prostitutes apparently having the same risk factors as the rest (same heterosexual male partners) have remained healthy and seronegative for varying durations of time? (Ngugi E.N.; Personal communication).

(b) Why does the transmission rate from male to female vary so markedly such as:

i) Low transmission rate of 6% among haemophiliac partners (75).

ii) Transmission rate of 16% among female partners of men with blood transfusion associated infection (76).

iii) High transmission rate of 35% among female partners of male intravenous drug users (76).

(c) Are there humoral or behavioural protective factors in some sexual partners?

(d) Are there certain heterosexual practices that tend to promote or reduce infection from an HIV-infected individual to the sexual partner?

It is with the above unanswered questions that a study to evaluate sexual partners of HIV-infected individuals and/or AIDS patients was designed.

The study may form the basis for counselling spouses in actual preventive measures that can be applied by those who remain uninfected.

It could also be the basis for the search of some protective factors, humoral, behavioural and genetic in the persistently seronegative spouses. Such factors it was thought, could be of further use in the attempts to prevent HIV-infection.

F - MATERIALS AND METHODS

Research Design:

The research was a cross-sectional survey carried out on some (not all patients) HIV-infected persons and their sexual partners (spouses) admitted at KHN medical wards.

Case selection:

Between February and October 1993 (inclusive) some patients admitted to the medical wards with suggestive signs and symptoms received pre-test counselling for screening for HIV-infection by ELISA.

Patients who turned seronegative received communication of the results of the test and were advised on methods of prevention of HIV-infection and excluded from the study.

The HIV-positive individuals received post-test counselling and were requested for their participation in the study. Their spouses were also counselled about the HIV-serological status of their partners and consent sought for recruitment into the study.

Inclusion and exclusion criteria:

HIV-infected index cases with their spouses with penile-vaginal heterosexual contact who gave consent to participate in the study were recruited. Only spouses with regular sexual intercourse for at

least one month with the HIV-infected index cases were included in the study.

HIV-infected patients and their spouses who declined consent were excluded from the study. Those living long distances from Nairobi were also excluded because of difficulties in follow up during the study. The Index cases together with their sexual partners were followed up prospectively for at least six months.

CASE DEFINITIONS

(a) **Index cases included:**

(i) **AIDS patient**: defined as an adult person initially identified to have AIDS according to the World Health Organization (WHO) clinical diagnosis criteria (appendix I).

The diagnosis of AIDS patients in the study were confirmed by a positive serological test by ELISA for HIV-antibodies once.

(ii) **HIV-infected individual**: Defined as an individual symptomatic or asymptomatic but whose analysis for HIV-antibody is positive (Appendix III).

(b) **Spouse (Sexual Partner):** Refers to a woman or man living with the Index case as wife or husband at the time of diagnosis of HIV-infection or AIDS in the Index case.

CLINICAL EVALUATION

Interview

At initial enrolment of the Index case and their sexual partner, démographic information, a medical history and a history of sexual practice were obtained in a standard questionnaire (Proforma-Appendix IV). Information sought included:- age; sex; marital status; residence; place of birth; other sexual partners; frequency of sexual intercourse per week; blood transfusion; use of condoms and reasons for use; intravenous drug use; injections at various health facilities and circumcision for males. In addition, a history of previous genital ulcer and/or sexually transmitted diseases (STDs) was obtained. Having more than one partner besides index case was considered as having 'multiple sexual' partners in the last ten years.

Clinical Examination:

All HIV-infected or AIDS patients with their sexual partners had physical examination and the clinical stage of HIV-1 infection was determined. The assessment of the clinical stage of disease was based on the parameters set out by Centres for Disease Control as has already been described (Appendix II).

LABORATORY INVESTIGATIONS:

Samples of blood were taken from each Index case and sexual partner for the following investigations:-

(i) 5 mls of blood in a plain bottle was taken for detection of HIV-antibody by Enzyme Linked Immunosorbent Assay (ELISA). HIV-antibody testing was done by ELISA as described (Appendix-III) using the Enzygnost anti-HIV-1/2 Kit [Behring Company, Paul-Ehrlich-Institut, Federal Agency for Sera and Vaccines (Germany)]. Western blot examination was omitted due to expense. In its place two separate ELISA screening tests were done on the same sample and confirmation was done by a third ELISA which employs a Recombigen antigen (env and gag) manufactured by the Cambridge Biotech Corporation of Britain (365 Plantation Street, Biotechnology Research park, Worcester MA 01605). Sexual partners who turned seronegative were retested again at 2,4,6 months interval from the day of recruitment.

(ii) 2 mls of blood was taken for full blood count and ABO - blood grouping.

Haemoglobin level, platelets and white cell count were determined using Coulter-Counter model S-Plus IV and V equipment.

Total lymphocyte counts with specific assessment of the CD4+ and CD8+ together with their ratios was determined using a flowcytometer-Facscan (Becton Dickinson Company, Florida, U.S.A.).

DATA MANAGEMENT AND STATISTICAL ANALYSIS

The data was entered in the database management system, FILEMANAGER and later converted to SPSS format. Data was stored in several files, for index cases and partners separately and laboratory data separate from demographics, behavioural and clinical data. Key variables identified the index cases and their partners. SPSS were used for both data management, and statistical analysis. For statistical questions concerning both index cases and partners, files were joined on the key variable, i.e. information regarding index cases was added to partner files using the JOIN MATCH procedure in SPSS.

Standard statistical methods were used for statistical analysis. For cross-classified counts, the CHI-SQUARE test and the Mantel-Haenszel trend test were used. For continuous variables, t-tests, either paired (e.g. for comparing CD4 counts of index cases and partners) or unpaired (e.g. for comparing CD4 counts of HIV positive to HIV negative partners). For more than two groups the MEANS procedure and Analysis of Variance were used.

Harvard Graphics 3 was used to produce bar graphs and similar figures.

F - RESULTS

During the study period between February and October 1993 (inclusive) 261 patients (not all the eligible patients admitted in the medical wards) were screened for HIV-antibody due to suggestive signs and symptoms. Ninety (34.5% of those screened) turned seronegative and were excluded from the study.

171 (65.5% of those screened) were HIV-positive and eligible for recruitment into the study. One hundred and twenty (70.2% of eligible index cases) HIV-infected individuals with their 123 spouses participated in the study. Excluded were 31 (18.1%) index cases whose spouses could not be traced, 15 (8.8%) index cases who declined consent and 5 (2.9%) index cases whose spouses declined participation.

(i) Results for the index cases:

Of the 171, eligible HIV-positive index cases, 120 (70.2%) participated in the study. The male to female ratio was 1.3:1.

Table 1 shows the age-sex distribution of the index cases.

(a) Age-sex distribution of index cases:

Table 1: Age-sex distribution of the index cases.

AGE-RANGE (YEARS)	MALES	FEMALES	TOTAL
20-24	0	17	17
25-29	10	15	25
30-34	16	9	25
35-39	15	6	21
40-44	12	3	15
45-49	10	2	4
50-54	4	0	0
55-59	0	0	0
60-64	1	0	1
TOTAL NOS (%)	68 (56.7)	52 (43.3)	120 (100.0)

Note: Numbers in brackets are in percentages.

Females have a higher distribution in the younger age group range (20-29 years) compared with the males (25-49 years). Females were statistically younger with a mean age of 28.4 years (S.D.=6.9) compared with the males with a mean age 37.5 years (S.D. = 7.7) (t-test; $p < 0.001$).

(b) Risks of HIV-infection for index cases:

Figure-1 shows the frequency distribution of index cases according to sex in relation to risk factors for HIV-infection.

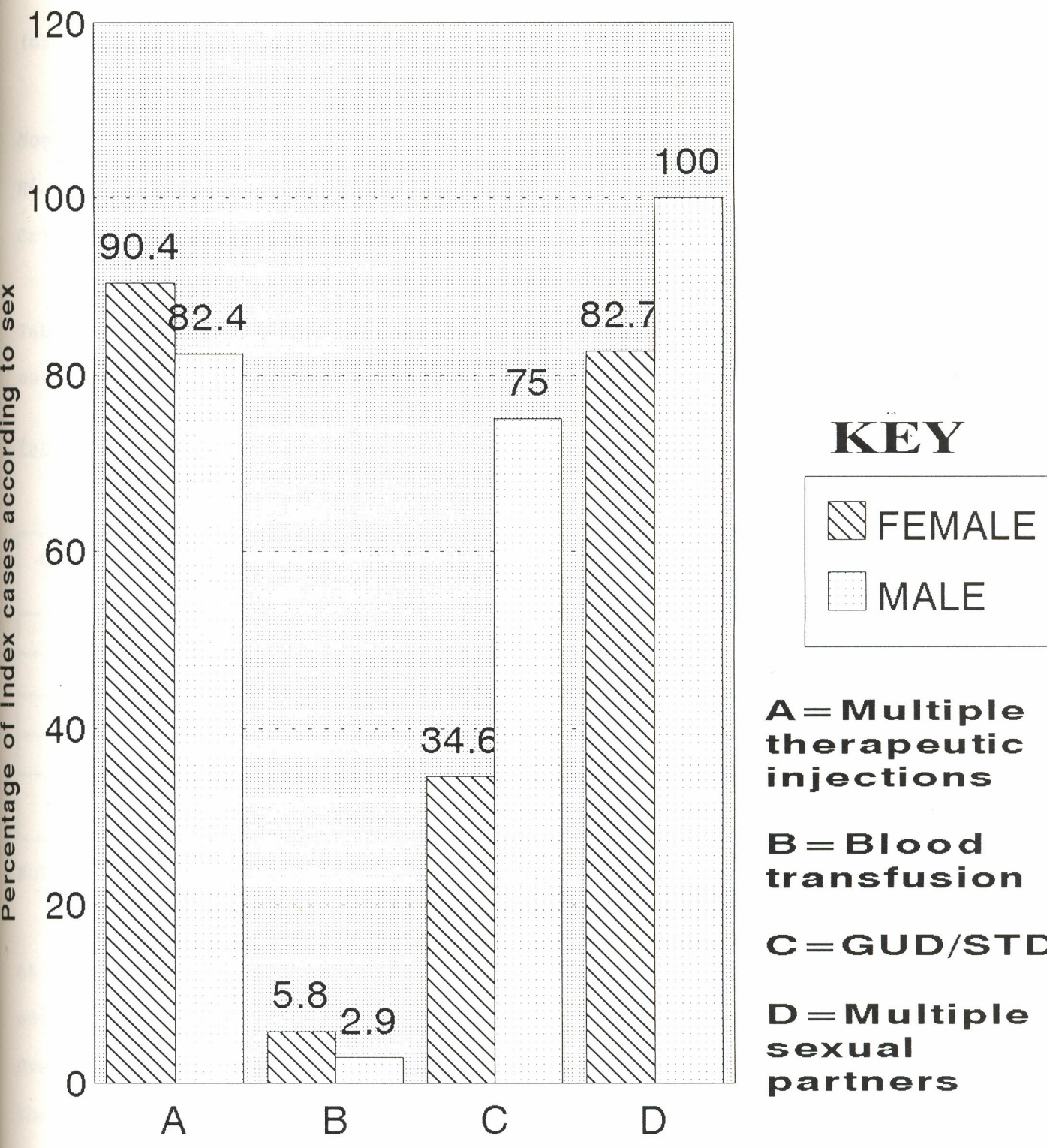
The factors explored include: history of multiple sexual partners, blood transfusion, intravenous drug use (IDU) and multiple injections in the last 10 years.

Of the 120 index cases, 68 males (100%) and 43 females (82.7%) reported a history of multiple sexual partners besides their spouses. Males had statistically higher prevalence of multiple sexual partners compared with the females (chi-square; $p < 0.001$). Nine index females denied a history of extra marital sexual intercourse and only one of them had blood transfusion in the last 10 years. All the 9 females had a history of multiple therapeutic injections. 75% of males and 34.6% of females reported a history of genital ulcer disease (GUD) or having been treated for an STD in the past 10 years. Males had a statistically higher prevalence of GUD and/or STD compared with the females ($p < 0.05$).

Only 2.9% male and 5.8% female index cases reported history of blood transfusion in the last 10 years. The females had blood transfusion following post partum haemorrhage. One male had blood transfusion following gastrointestinal bleeding and the other after a road traffic accident.

Of the 120 index cases 82.4% of the males and 90.4% of the females reported history of having had multiple therapeutic injections at various health facilities in the last 10 years. There was no statistical significant difference between the sexes ($p > 0.05$). No index case reported a history of intravenous drug use in the last 10 years.

Of the 68 index males, 34 (50%) were circumcised and 34 (50%) were not circumcised.



ig 1
Distribution of index cases according to sex and associate
isk factors for HIV infection

(c) **Clinical Status and the outcome of the index cases during the study**

Most of the index cases presented with AIDS according to the World Health Organization and Centres for Disease Control Classification Criteria - (Appendices I and II).

Table 2 shows the correlation of the clinical stage with the outcome of the index cases during the study.

Table 2: Correlation of clinical stage and outcome of the index cases.

CLINICAL STAGE	OUTCOME			TOTAL
	ALIVE	DEAD	LOST TO FOLLOW UP	
1	0	1	0	1 (0.8)
2	10	0	4	14(11.7)
3	7	5	18	30(25.0)
4	9	41	25	75(62.5)
TOTALS	26 (21.7)	47 (39.2)	47 (39.2)	120 (100.0)

NOTE: Numbers in brackets are percentages.

At recruitment 75 (62.5%) index cases had AIDS (stage 4) clinically and by the end of the study 41 (54.7%) of them were dead.
Over all at least 47 (39.2%) of the index cases died by the end of the follow-up of AIDS related complications.

(d) Correlation of clinical stage of index cases with HIV-serological status of their spouses:

Table 3 shows the correlation of clinical stage of index cases with HIV-serological status of their spouses.

Index cases who had clinical stage 3 were married with 17 (19.5% of HIV-positive) spouses and 13 (36.1% of HIV-negative) spouses. Index cases who had clinical stage 4 were married 59 (67.8% of HIV-positive) spouses and 17 (47.2% of HIV-negative) spouses. There is no statistically significant association between the clinical stage of HIV-infected index cases and the HIV-serological status of their spouses in the study ($p > 0.05$).

Table 3: Clinical stage of index cases correlated with HIV-serological status of their spouses:

	SPOUSES		TOTAL SPOUSES
INDEX CASE CLINICAL STAGE	HIV-POSITIVE	HIV-NEGATIVE	
1	2	1	3 (2.4)
2	9	5	14 (11.4)
3	17	13	30 (24.4)
4	59	17	76 (61.8)
TOTAL	87 (70.7)	36 (29.3)	123 (100.0)

N.B. Numbers in brackets are percentages

(ii) Results for the sexual partners (spouses)

Through the 120 index cases, 123 sexual partners were recruited into the study. One male index case had 2 wives and another 3 wives accounting for the 123 sexual partners. Of the 123 sexual partners 51 were males and 72 were females giving male-female ratio of about 1:1.4.

Age - sex and serological status of the sexual partners

Of the 51 male spouses, 64.7% were HIV-positive and of the 72 female spouses 75% where HIV-positive. There was no significant difference between the sexes in terms of HIV-seropositivity ($p > 0.05$). Table 4 shows the mean age, sex and serological distribution of the sexual partners.

Table 4: Mean age, sex and serological status of the sexual partners

Serological status and sex	Cases	Percentage (%)	Mean age (years)	S.D
HIV-Positive	87	70.7	31.1	8.0
Male	33	26.8	34.7	8.7
Female	54	43.9	28.9	6.6
HIV negative	36	29.3	32.2	7.3
Male	18	14.6	35.2	5.5
Female	18	14.6	30.9	8.4

Over all there is no statistically significant difference between the mean age in years of HIV-positive [31.1 (S.D. = 8.0)] and seronegative [32.2 (S.D. = 7.3)] sexual partners. Female spouses

have a significant younger mean age in years [29.4 (S.D. = 7.1)] compared with male spouses [35.0 (S.D. = 7.7)] (t-test; $p < 0.001$).

Areas of residence of sexual partners in Nairobi

Table 5 shows the distribution of present area of residence of sexual partners within sublocations in Nairobi. 25 sexual partners lived outside Nairobi; that is from neighbouring districts of Kiambu, Kajiado, and Machakos. 8 of these were seronegative for HIV-antibodies.

Most of the HIV-positive sexual partners lived in the slum areas of Nairobi; Dandora 11.5%; Kariobangi 9.2%; Kibera 8.0%; Eastleigh 6.9%; Mathare 5.7% and Huruma 4.6%.

Table 5: Present areas of residence of sexual partners in Nairobi and other areas and their serological status:

REGION IN NAIROBI	HIV-POSITIVE	HIV-NEGATIVE	TOTAL
DANDORA	10	6	16
KIBERA	7	4	11
KARIOBANGI	8	1	9
HURUMA	4	4	8
EASTLEIGH	6	1	7
MATHARE	5	1	6
DAGORETI	1	2	3
NBI-SOUTH	2	0	2
MARINGO	2	0	2
LANGATA	2	0	2
KAWANGWARE	2	0	2
RIRUTA	1	1	2
PUMWANI	2	0	2
NGOMONGO	1	1	2
MUTHAIGA	1	1	2
KAHAWA	2	0	2
MBOTELA	1	1	2
MAJENGO	1	0	1
KARIODUDU	1	0	1
KENYATTA ESTATE	0	1	1
JERICHO	0	1	1
MUTHURUA	1	0	1
EMBAKASI	0	1	1
KILIMANI	1	0	1
UMOJA	1	0	1
NGEI ESTATE	1	0	1
PANGANI	1	0	1
ZIWANI	1	0	1
WAITHAKA	1	0	1
KALOLENI	1	0	1
SHAURI MOYO	1	0	1
*OTHERS	17	8	25
TOTAL	87	36	123

*OTHER - Refers to areas outside Nairobi

Relationship and sexual practice for the sexual partners with index cases

All the 123 sexual partners were related with the index case as spouses (married couples). The mean duration of marriage for all HIV-positive spouses was 9.0 (S.D. = 7.4) years and for the seronegative partners was 9.7 (S.D. = 7.3) years. There is no significant difference between the 2 groups (t-test; $p > 0.05$). However among the 36 HIV-negative spouses (male-female ration 1:1), females had a significantly longer mean duration of marriage of 12.6 (S.D.= 8.5) years compared with the seronegative male spouses of 7.1 (SD = 4.9) years (t-test, $p < 0.05$).

The mean time span from last sexual contact with index case to day of recruitment for HIV-positive spouses was 77.9 (S.D = 81.5) days and for the HIV-negative spouses was 74.9 (S.D = 82.2) days. There is no significant difference between the two means ($p > 0.05$).

Table 6 shows the frequency of sexual intercourse of sexual partners with index cases in relation to HIV-serological status. 44.8% of HIV-positive spouses and 47.3% of HIV-negative spouses had sexual intercourse with index cases once per week or less than twice per month. 55.2% of HIV-positive spouses and 52.7% of HIV-negative spouses had sexual intercourse with index cases at least twice per week ($\geq$ 2/week).

Table 6: Frequency of sexual intercourse of sexual partners with index case in relation to HIV-serological status.

FREQUENCY OF SEXUAL INTERCOURSE	HIV-POSITIVE	HIV-NEGATIVE	TOTAL
Less than twice/month	20	11	31
Once per week	19	6	25
Twice per week	29	9	38
More than twice per week	19	10	29
TOTAL	87	36	123

There was no significant difference between frequency of sexual intercourse with index cases between the HIV-seropositive and the seronegative sexual partners ($p > 0.05$). When the rate of sexual intercourse of spouses with index cases was controlled for sex, the HIV-positive male spouses were not statistically different from the seronegative males ($p > 0.05$), and the HIV-positive female spouses were not statistically different from the seronegative females ($p > 0.05$).

Other possible risk factors of infection of sexual partners besides the index case:

(1) Other multiple sexual partners: Heterosexual contact with one or more other sexual partners besides the index case was taken as multiple other sexual contacts in the last 10 years.

Table 7 shows the correlation between other heterosexual contacts of spouses in relation to their HIV-serological status. Of the 51 male spouses, 33 were HIV-positive and 18

were HIV-negative. 90.9% of the HIV-positive male spouses and all the HIV-negative (100%) gave a history of multiple other sexual partners. There is no statistically significant difference between the 2 groups ($p > 0.05$).

Only 3 (5.9%)males denied a history of other heterosexual contacts besides their wives.

Of the 72 female spouses, 77.8% (of 54 HIV-positive) and 38.9% (of 18 seronegative) gave a history of multiple sexual partners. On the other hand 22.2% (of HIV-positive) and 61.1% (of seronegative) spouses denied a history of multiple sexual partners. There was a statistically significant difference between the seronegative and seropositive female spouses in relation to multiple sexual partners (p-value = 0.002).

Table 7: Frequency distribution of other heterosexual contact of sexual partners in relation to serological status:

SPOUSES' SEX	OTHER MULTIPLE SEXUAL CONTACTS	HIV-POSITIVE	HIV-NEGATIVE	TOTAL
MALE	Yes	30	18	48
	No	3	0	3
FEMALE	Yes	42	7	49
	No	12	11	23
TOTAL		87	36	123

2. Multiple therapeutic injections and accidental injections with contaminated needles:

Table 8 shows frequency distribution of multiple therapeutic injection of sexual partners in relation with serological status and also other risk factors for HIV-infection. 74 seropositive partners reported having had multiple intramuscular injections for a variety of ailments in the past 10 years. 28 seronegative partners had a similar history. Only 21 sexual partners denied a history of past multiple injections and 8 of them were seronegative. There was no significant difference between the seropositive and seronegative partners in terms of these injections ($p > 0.05$). No partner reported having had accidental injections with contaminated needles.

(3) Blood transfusion and intravenous drug use (Table 8)

Only 7 spouses reported having received blood transfusion in the last 10 years. 6 of them were females of whom 2 were HIV-negative. Of these seronegative, one received blood after delivery and another after severe malaria. Of the 4 seropositive females, one

received blood after an abortion in a provincial hospital, 1 after a caesarean section in a provincial hospital and the other 2 after delivery at Pumwani Maternity. The only seropositive male received blood after a road traffic accident at a district hospital.

No sexual partner gave a positive history of intravenous drug use.

Table 8: Other possible risks for infection for sexual partners besides index case.

RISK	Response	HIV-Positive	HIV-Negative	Total Nos (%)
Other	Yes	72	25	97(78.9)
Multiple sexual Partners	No	15	11	26(21.1)
Multiple	Yes	74	28	102(82.9)
Therapeutic injections	No	13	8	21(17.1)
Blood	Yes	5	2	7(5.7)
Transfusion	No	81	35	116(94.3)

N.B. Numbers in brackets are percentages

1. Genital ulcer disease as a risk factor for HIV-infection of spouses

Table 9 shows the correlation of history of genital ulcer disease and other venereal disease (GUDVD) of spouses in relation to HIV-serological status.

Over all, 52 (42.3%) sexual partners gave history of past genital ulcer disease and other venereal diseases and 42 (80.8%) of these were HIV-positive, 10 (19.2%) were seronegative. The difference between seronegative and seropositive sexual partners is not statistically significant ($p = 0.057$).

Controlled for sex, of 33 HIV-positive males 72.7% accepted having been treated for sexually transmitted diseases in the past, 27.3% denied such a history. 8 seronegative males had a history of GUDVD but 10 denied such a history. There was statistically significant difference between seropositive and seronegative males as regards GUDVD ($p = 0.045$). Of the 54 HIV-positive female spouses, 33.3% accepted history of GUDVD and 66.7% denied such a history. Of the 18 seronegative female spouses, 16 (88.9%) denied history of GUDVD and only 2 (11.1%) gave a history of GUDVD. The statistical difference between seropositive and seronegative females in relation to GUDVD was not statistically significant ($p = 0.068$).

Table 9: Frequency distribution of history of genital ulcer disease and other venereal diseases (GUDVD) in relation to serological status of sexual partners.

SEX	RISK	RESPONSE	HIV-POS.	HIV-NEG.	TOTAL
Male	GUDVD	Yes	24	8	32
		No	9	10	19
Female	GUDVD	Yes	18	2	20
		No	36	16	52
		TOTAL	87	36	123

Note GUDVD = Genital ulcer disease and other venereal diseases.

5. Lack of male circumcision as a risk factor for HIV-infection

Table 10 shows the frequency distribution of the studied population in terms of male circumcision. Of the 68 male index cases the ratio of those circumcised to those not circumcised was 1:1. Of the 51 male sexual partners, only 12 were not circumcised and of these 10 were HIV-positive and 2 seronegative. Of the 39 circumcised, 16 were seronegative and 23 seropositive. There was no statistical significant difference between the two groups in relation to -HIV-serological status (Chi-square test; $p = 0.123$).

The male index cases were matched with their female sexual partners and the latter grouped according to HIV-serological status. 34 circumcised index cases were related to 28 seropositive females and 9 seronegative females. A similar number of uncircumcised index cases related to 26 seropositive females and 9 seronegative females. There was therefore no statistically significant difference in the seropositive and seronegative female sexual

partners of male index cases grouped according to state of circumcision ($p > 0.05$).

Table 10 Frequency distribution of the studied population in terms of male circumcision in relation to serological status:

SEX	CIRCUMCISION STATE	NOS. HIV-POS	NOS. HIV-NEG
Male Index case	circumcised	34	0
	not circumcised	34	0
Male Sexual Partners	circumcised	23	16
	not circumcised	10	2
Female Sexual Partners of	circumcised index cases	28	9
	not circumcised index cases	26	9

Condom use as a protection for HIV-infection

Enquiries were made from couples whether they used condoms. Table 11 shows frequency of previous condom use. 74 HIV-positive and 26 seronegative sexual partners did not use condoms at all. 13 seropositive and 7 seronegative spouses used condoms occasionally for the purpose of family planning.

Two seronegative male sexual partners reported use of condoms regularly. One male partner had been using condoms with seropositive wife and he had remained seronegative for the 6 months of follow up. One other male had been using condoms for the last 5 years with wife for the purpose of family planning. When he learned that the wife had tested positive for HIV-infection, he abstained from sexual intercourse with her.

Table 11: Frequency of previous use of condoms by couples in relation to serological status:

CONDOM USE FREQUENCY	HIV-POS.	HIV-NEG.	TOTAL
Not used at all	74	26	100
Occasionally	13	7	20
Always	0	2	2
Total	87	36	123

The proportion of spouses who used condoms regularly was too small and insignificant.

linical staging of the sexual partners according to CDC and WHO lassification

able 12 shows the distribution of sexual partners according to linical stage and final outcome during the study. Seventy nine 64.2%) sexual partners were asymptomatic (stage 1) at recruitment nd 36 of these were lost to follow up. At least 2 sexual partners one stage 2 and the other stage 4) died by the end of 6 months ollow up.

able 12: Distribution of sexual partners according to clinical stage and final outcome:

CLINICAL STAGE	OUTCOME			
	ALIVE	DEAD	LOST TO FOLLOW UP	TOTAL NOS.(%)
1	43	0	36	79(64.2)
2	19	1	18	38(30.9)
3	2	0	1	3(2.4
4	2	1	0	3(2.4)
TOTAL	66(53.7)	2(1.6)	55(44.7)	123(100.0)

Note - Numbers in brackets are in percentages.

HIV-Seronegative sexual partners

Table 13 shows the distribution of HIV-negative spouses according to age, sex, clinical stage, serial HIV-serological status, absolute CD4+ T-lymphocyte counts, CD8+ T-lymphocyte counts and their ratios.

Of the 36 seronegative partners, (29.3% of spouses) the male to female ratio was 1:1. Mean age in years for the males was 35.2 (S.D = 5.5) and for females it was 30.8 (S.D = 8.4); no statistical difference was observed between the two means. All the seronegative partners were asymptomatic (clinical stage 1).

None of the seronegative partners who offered themselves for retesting seroconverted during the follow up. 13 were retested at 2 months, 12 at 4 months and 10 at 6 months. One seronegative partner (number 20) who was tested once had absolute CD4+ T-lymphocyte count of 184/mm^3, significantly low (normal range 380-1080/mm^3) and a CD4+: CD8+ ratio of 0.20. One would have liked to retest this sexual partner but she was lost to follow up.

Seronegative spouse No. 14 with CD4: CD8 -ratio of 0.30 had absolute CD4+ T-lymphocyte counts 713/mm^3 (within normal range) but significantly elevated absolute CD8+ absolute counts of 1686/mm^3 (normal range 370 -1010/mm^3).

Seronegative partners numbers 7,9,14,19,22,30 and 36 had CD4+: CD8+ ratios on the lower side (normal range 0.6-1.6).

Table 13: SERONEGATIVE SPOUSES, AGE, SEX, CLINICAL STAGE, SERIAL HIV-TESTS; ABSOLUTE CD4+ AND CD8+ CELLS AND RATIOS:

NO	AGE YRS.	SEX M OR F	CLINI-CAL STAGE	HIV-TEST ELISA 0Mo	2Mo	4Mo	6Mo	CD4+ Absolute N=380-1080/mm³	CD8+ Absolute N=370-1010/mm³	CD4 CD8 RATIO (N=0.6-1.6)
1	47	F	1	-	-	NT	N	501.12	556.88	0.90
2	30	M	1	-	-	NT	-	464.94	757.68	0.60
3	36	M	1	-	NT	NT	NT	NT	NT	NT
4	48	M	1	-	-	-	NT	610.56	661.44	0.90
5	21	F	1	-	NT	NT	NT	NT	NT	NT
6	44	M	1	-	NT	NT	-	498.96	554.40	0.90
7	33	M	1	-	NT	-	-	619.75	1289.08	0.50*
8	41	F	1	-	NT	NT	NT	NT	NT	NT
9	32	M	1	-	NT	NT	-	995.28	1887.60	0.50*
10	35	M	1	-	NT	NT	NT	NT	NT	NT
11	27	F	1	-	NT	NT	NT	NT	NT	NT
12	28	F	1	-	-	NT	-	993.30	669.90	1.50
13	21	F	1	-	NT	NT	NT	661.98	561.68	1.20
14	37	M	1	-	NT	NT	NT	713.46	1686.36	0.30*
15	32	F	1	-	NT	-	NT	1041.18	718.91	1.40
16	40	F	1	-	NT	NT	NT	927.36	463.68	2.00
17	31	M	1	-	NT	-	-	548.68	756.80	0.70
18	37	M	1	-	NT	NT	NT	748.80	1146.60	0.70
19	25	M	1	-	NT	-	-	573	1102.00	0.50*
20	37	M	1	-	NT	NT	NT	184.24	815.92	0.20*
21	22	F	1	-	NT	-	NT	622.34	772.56	0.80

Note: This table continues on the next page.

Table 13: continued

NO	AGE YRS.	SEX M OR F	CLINI-CAL STAGE	HIV-TEST ELISA 0Mo	2Mo	4Mo	6Mo	CD4+ Absolute N=380-1080mm^3	CD8+ Absolute N=370-1010/mm^3	CD4 / CD8 RATIO (N=0.6-1.6)
22	35	F	1	-	-	-	-	623.70	1155.00	0.50*
23	36	F	1	-	-	-	-	641.52	524.88	1.20
24	40	M	1	-	NT	NT	NT	901.32	876.96	1.00
25	32	M	1	-	NT	NT	NT	708.48	970.85	0.70
26	23	F	1	-	-	-	NT	1124.24	684.32	1.60
27	35	M	1	-	-	-	NT	422.40	456.40	1.20
28	25	F	1	-	NT	NT	NT	817.70	889.85	0.90
29	36	F	1	-	-	NT	NT	1008.00	831.60	1.20
30	29	M	1	-	NT	NT	NT	622.44	1149.12	0.50*
31	39	M	1	-	-	-		719.78	1116.90	0.60
32	44	F	1	-	NT	NT	NT	475.20	792.00	0.60
33	26	F	1	-	NT	NT	NT	1048.32	725.32	1.40
34	30	F	1	-	-	-	-	752.76	885.60	0.80
35	22	F	1	-	NT	NT	NT	1057.05	1275.75	0.80
36	38	M	1	-	-	-	NT	415.80	1148.40	0.40*

Key: M = Males
F = Females
N = Normal range
NT = Not tested
MO = Months

(-) = Elisa Negative for HIV
* Low CD4+/CD8+ ratio

Case Reports:

Below are case reports of 2 seronegative spouses who despite regular sexual intercourse with their partners (index cases) have remained seronegative in the six months of follow-up.

Case I

A 36 year old HIV-negative female got married to a salesman 13 years ago. She denied having had extra-marital sexual relationship; her HIV-positive husband admitted having had multiple sexual relationships out of marriage. She last had coitus with husband 2 months before recruitment. The husband later died of intractable diarrhoea at K.N.H. The wife has remained seronegative for the last 9 months and is being followed up at K.N.H outpatients clinic.

Case II

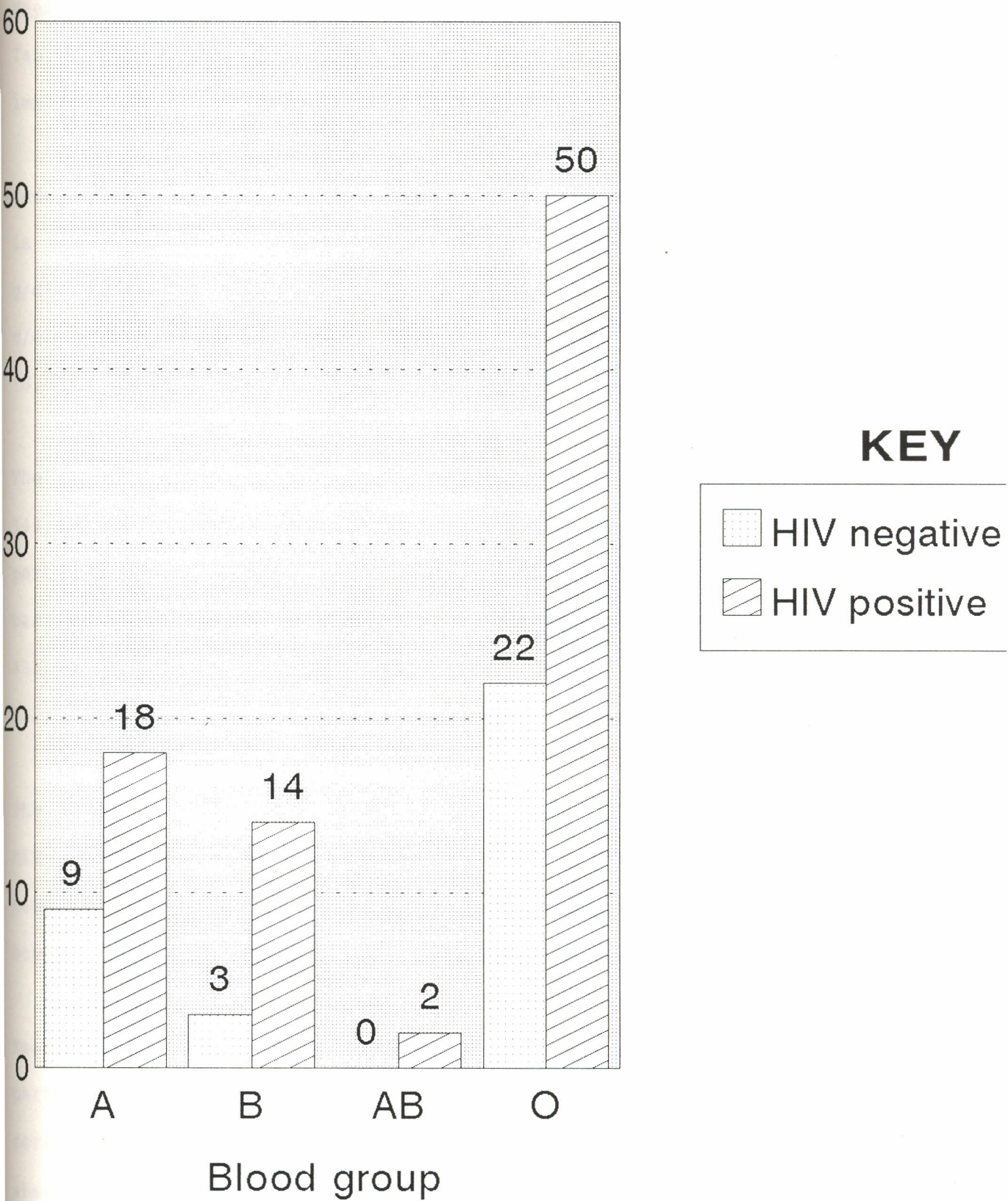
39 year old HIV-negative male works for the Ministry of Lands as a soil conservationist. He lived with the first wife from 1980-1986 when they had one child. In 1986 he divorced her because of domestic problems. In 1988, he married a second wife and admits he was not sure of her past sexual history. The wife admits having had at least 2 other heterosexual contacts before marriage but thereafter she had no other sexual partners besides the husband. They got their first child who died of chest problems at 2 months of age. Their second child died at 3 months and the third died at K.N.H at 2 months. The mother (HIV-positive) of the children died later of chest problems at a District hospital. The husband has remained seronegative for the last 9 months. He is currently being

followed up at K.N.H outpatient clinic.

Laboratory Results

ABO - blood group in relation to HIV-serological status of the sexual partners

Figure 2 shows the bar graph distribution of the frequency of the blood groups for the sexual partners in association with their serological status. 118 sexual partners had their blood group tested. The blood group for 5 spouses could not be ascertained because of various technical reasons such as loss of results in the laboratory. Twenty seven (22.9%) had blood group A, 17 (14.4%) group B, 2 (1.7%) group AB and 72 (61.1%) group O. None of the sexual partners was Rhesus factor negative. There was no statistically significant difference between the seronegative and seropositive sexual partners in relation to the 4 blood groups (A, AB, B and O) compared together (Chi-square test; $p = 0.889$).



uency Distribution of ABO blood group in relation to serological status

ematological and immunological parameters.

ble 14 shows a summary of mean values of some haematological and mmunological parameters of the studied population.

dex cases had a mean haemoglobin of 11.1 (S.D. = 2.4) g/dl, which lower than that for the sexual partners of 13.6 (S.D. = 2.0) dl. The HIV-positive spouses had a mean of 13.2 (S.D. = 1.9) dl and the seronegative spouses had the highest mean of 14.6 .D. = 1.7) g/dl.

e index cases had a mean absolute CD4 + T-lymphocyte count of 3/mm^3 which is significantly lower than that for the sexual rtners of 524/mm^3 (t-test; $p < 0.001$). Amongst the sexual rtners, those seropositive had mean CD4 + lymphocyte counts of 2/mm^3 which is significantly lower than for the seronegative of 12/mm^3 (t-test; $p < 0.001$).

he mean absolute CD4+ T-lymphocyte count for the index cases of IV-positive sexual partners of 118/mm^3 is lower than that of index ases of HIV-negative sexual partners of 198/mm^3. The two means are owever not significantly different (t-test; $p = 0.055$).

lean absolute CD8+ T-lymphocyte count for the seropositive sexual partners (1326/mm^3) is significantly higher than that for those seronegative of 864/mm^3 ($p < 0.05$) and the index cases with an absolute mean of 802/mm^3 ($p < 0.05$).

Table 14: Mean Haematological parameters for the studied population

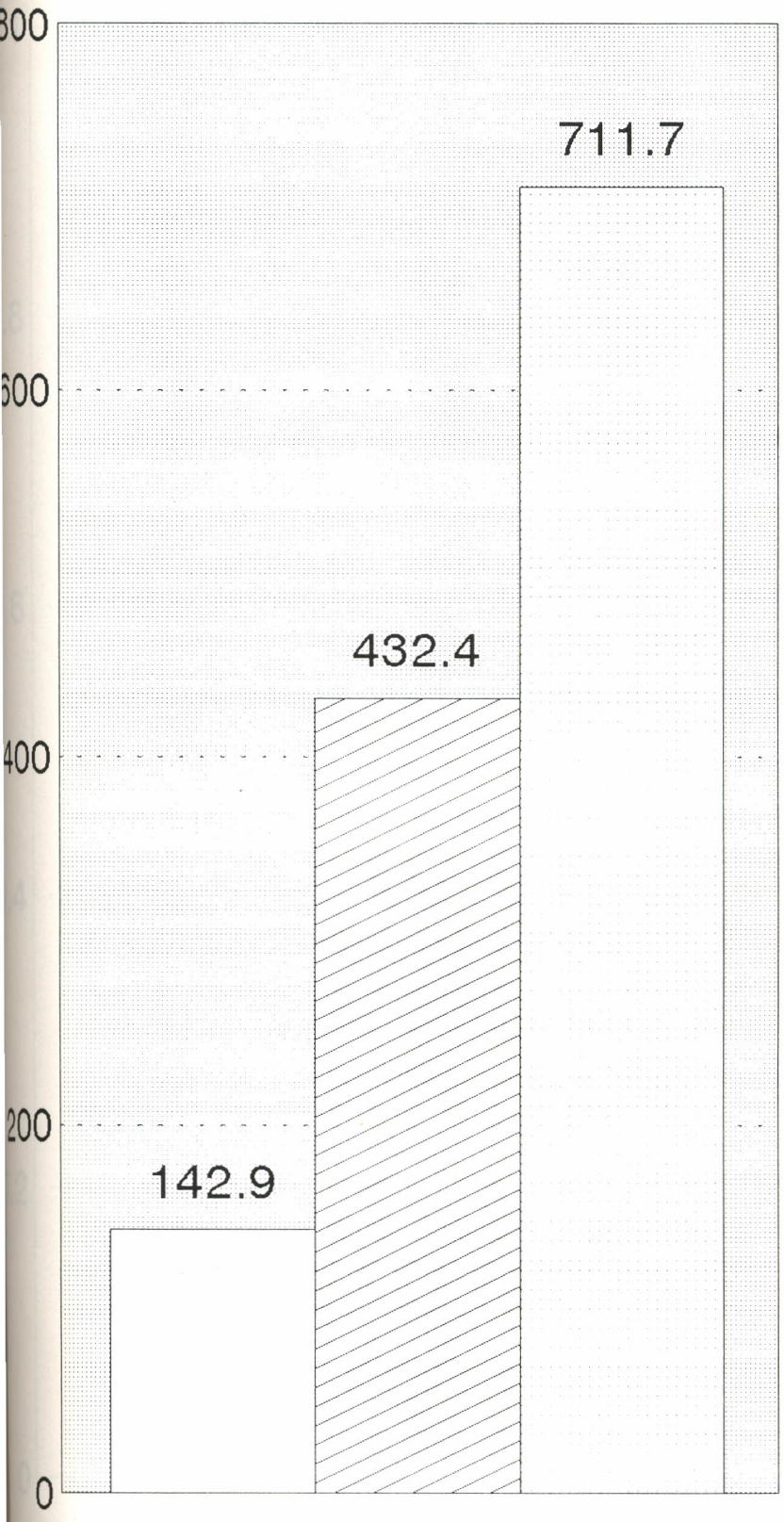
Population	Mean WBC/mm^3 4000-10000	Mean Hb g/dL N=12.0-18.0	Mean PLTSx$10^3/mm^3$ N=140-440	Mean CD4+ Count/mm^3 N=370-1080	Mean CD8+ Count/mm^3 N=370-1080	CD4+/CD8+ N=0.6-1.6
Index cases	8077	11.1	354	143	802	0.16
Index cases of HIV-Positive sexual partners	7593	11.0	356	118	801.	0.13
Index cases of HIV-Negative sexual partners	9170	11.3	348	198	803	0.23
All sexual partners	6123	13.6	290	524	1189	0.53
HIV-Positive sexual partners	6169	13.2	290	432	1326	0.37
HIV-Negative sexual partners	6017	14.6	291	712	864	0.90

N = Normal Range

Figures 3 and 4 show the frequency distribution of the absolute counts of CD4+ T-lymphocyte and CD4+: CD8 + ratios respectively of the studied population. The index cases had the lowest mean CD4+ cell counts and CD4+:CD8+ ratio; while seronegative sexual partners had the highest. There is a significant difference in variance of CD4+ cell counts between index cases and spouses (t-test; $p < 0.001$) and between the HIV-negative and seropositive spouses (t-test $p < 0.001$) (Table 13). The same applies for their CD4/CD8 ratios.

Table 15 shows the frequency distribution of the studied population according to CDC classification by levels of CD4 T-lymphocytes. Of the 120 Index cases, 89 (74.2%) had absolute CD4+ T-lymphocytes tested. 66 (74.2% of those tested) had absolute CD4+ T-lymphocyte count less than 200 cells/ml. Of the 99 sexual partners (80.5% of spouses) whose absolute CD4+ T-lymphocytes were tested, 79.8% had counts above 500 cells/ml, 17.2% had counts 200 - 499 cells/ml (inclusive) and 3% less than 200 cells/ml.

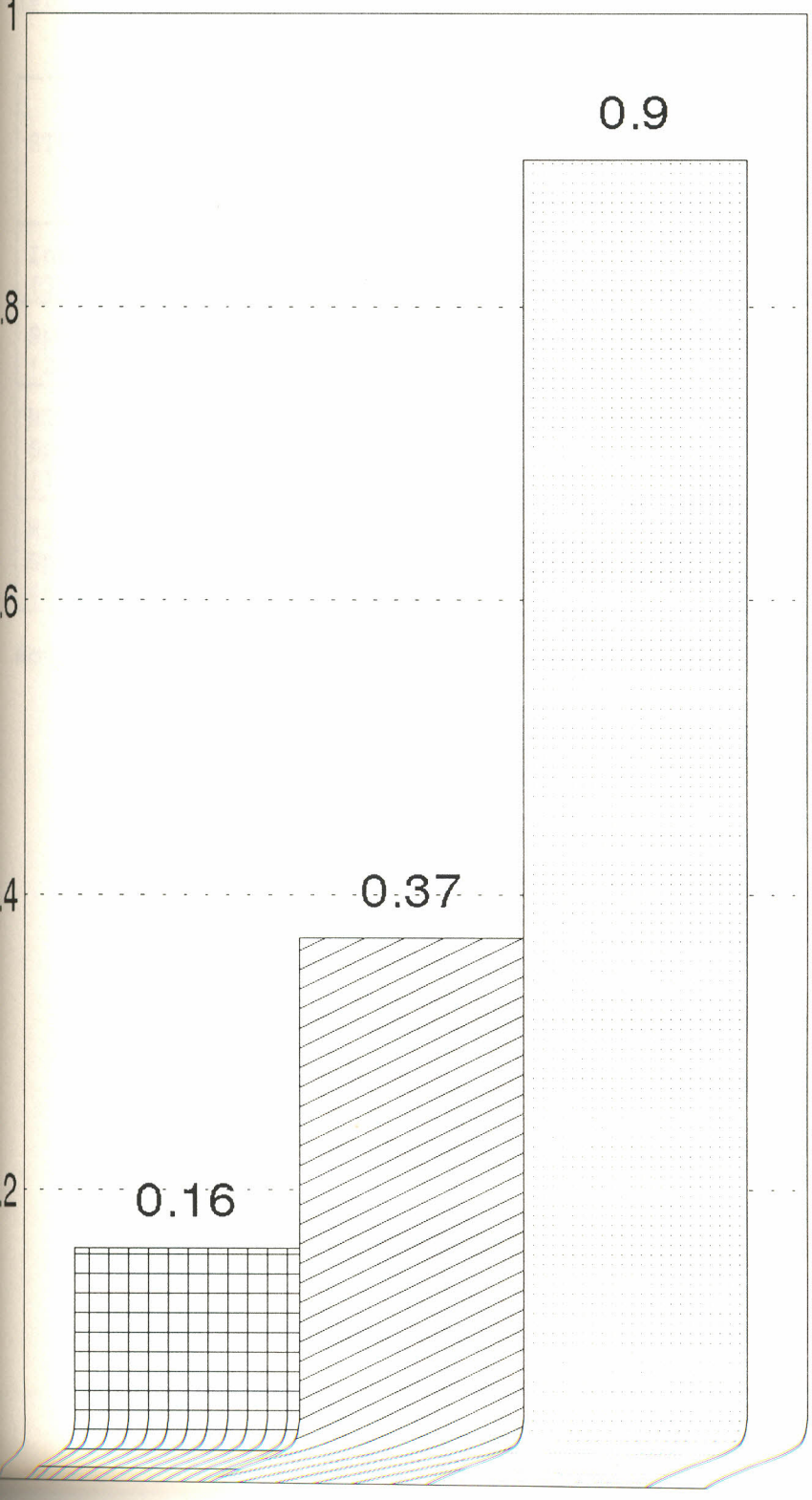
Of 28 seronegative sexual partners who had their CD4+ cells tested only 2 had counts in the 200-499/ml range. The rest had counts above 500 cells/ml.



KEY

- INDEX CASES
- HIV POSITIVE PARTNERS
- HIV NEGATIVE PARTNERS

n CD4 T-lymphocyte aboslute counts for the studied population



KEY

- INDEX CASES
- HIV POSITIVE PARTNERS
- HIV NEGATIVE PARTNERS

Table 15: CDC classification of the studied population according to CD4+ T-lymphocyte category levels.

STUDY GROUP	Index/Spouses whose CD4+ values were tested	Category I > 500 cells/ml	Category II 200- 499 Cells/ml	Category III < 200 cells/ml
Index cases (31 missing)	89	5	18	66
Spouses (24 missing)	99	79	17	3
HIV-Positive Spouses (17 missing)	70	52	15	3
HIV-Negative Spouses (8 missing)	28	26	2	0

Note: missing - are those whose CD4+ cells were not tested.

G - DISCUSSION

Demography and HIV-serological status:

In this study, the male to female ratios for the HIV-infected index cases (1.3:1) and for the spouses (1:1.4) were similar to those of other studies on heterosexual transmission of HIV done in Zaire and Zambia (43, 44). The ratios are however dissimilar to that found in western countries (10:1) where homosexuality and intravenous drug abuse are the main modes of transmission of HIV (27,28).

The overall HIV-seroprevalence among spouses in this study was 70.7%, confirming a higher heterosexual transmission rate of HIV in our environment compared to those of similar studies done by Fischl et al (14) at Miami, U.S.A.(58% among 45 spouses) and Marowa et al (26) in Zimbabwe (60% of 75 female spouses). The reasons behind the discrepancies in the seroprevalence of heterosexual transmission of HIV in different populations are not known. Bias in index case selection, different sample sizes and some genetic factors may have a role in the differences.

HIV infection is mainly a disease of sexually active young to middle aged individuals who are at their prime productive period of life (47,48). In this study it was observed that for both index cases and their HIV-positive spouses, females were significantly younger than males. The trend has been described by most studies done in Sub-saharan Africa (43-45).

The discrepancy in age between the males and females as regards HIV-infection may be explained by the fact that females achieve sexual maturity while much younger than the males. Literature reviewed show that, worldwide, fertility among female teenagers seem to be on the increase. Determinants include early sexual maturity, decreasing age of menarche and breakdown in cultural bonds (77). This was confirmed by a study carried out by Rogo et al (78) among secondary school girls (of all Provinces except Nairobi and North Eastern) in which the mean age of menarche was 14.4 $\pm$ 2.45 years. Similar cases of earlier female sexuality were also reported by Diejomao et al (79), in Nigeria. Decrease in the age of menarche has positively been correlated with increase fertility among females and the ability to conceive. Sexual maturity at an earlier age among females could predispose them to HIV-infection at a younger age compared with the males.

The areas of residence of HIV-positive spouses in Nairobi include the slums (collective 46%), neighbouring districts (Kiambu, Machakos, Kajiado and others) with 20% and the rest (34%) thinly spread in other sublocations in the city. This may at face value suggest a high concentration of HIV-infected individuals in the slums, but the most likely reason is that these could be the main catchment areas for Kenyatta National Hospital which is a low cost health facility in the City of Nairobi.

Relationship and sexual practices of the sexual partners and index cases

All the sexual partners recruited in the study had a spousal (marriage) relationship with the HIV-positive index cases. It was difficult during the study to recruit sexual partners who had casual sexual intercourse (male friends, female friends) with the index cases.

In common with a number of other studies, no relationship was found between the transmission probability of HIV among spouses and either duration of marriage (in years) or estimated frequency of sexual intercourse per week with index cases (80,81). Possible explanations for these findings include heterogeneity in either the HIV infectivity of the index cases or the susceptibility of their sexual partners. This is evidenced by some case reports of transmission of HIV occurring after one or few acts of sexual intercourse (80,82). In some other cluster studies, one individual has ben found to infect a high proportion of successive sexual partners (83).

Other risk factors for HIV-infection of spouses besides index cases:

(i) Multiple sexual partners, genital ulcer disease and other STDs:

Multiple heterosexual partners have been documented by several studies as an independent risk factor for HIV-infection and its spread (43,47,49).

In this study the HIV-positive male spouses (90.3%) and all their seronegative counter parts (100%), gave a history of having had multiple sexual partners resulting in no statistical difference between the two groups as regards HIV infection. On the other hand HIV-positive female spouses (77.8%) gave significant history of multiple sexual partners compared with their seronegative counter parts (38.9%).

The fact that male spouses were more involved with the risky behaviour of multiple sexual partners compared with the female spouses, yet their (males') HIV-seroprevalence (64.7%) is lower than that of females (75%) may suggest a less efficient female to male heterosexual transmission rate of HIV compared with male to female transmission. The two proportions, however, are not statistically different. The results of this study cannot exclude the possibility of heterosexual infection of spouses from partners other than the index cases.

Evidence from studies elsewhere suggests that the prevalence of other STDs [genital ulcer disease (GUD) independently] may increase the infectivity of index cases and the susceptibility of their spouses (48,50-51). In this study there was a relationship between history of STDs in the last 10 years and the transmission probability of HIV among male spouses, but there was no relationship among females spouses. The presence of genital ulcers and other STDS have been postulated to enhance acquisition of HIV-1 infection as the ulcers may provide the portal of entry for the virus (50,51). However it has been suggested that the presence of genital ulcers in males precludes sexual intercourse because of pain (84), but the experience of the Zimbabwe group (26) notes that men with lesions of recurrent genital herpes, superficial erosions of glans penis due to candidiasis continue to have sexual relationships.

The discrepancy between sexes found in this study may be due to the fact that some STDs such as chlamydial and gonococcal infections are more asymptomatic in most females compared with males. This could contribute to false negative responses among female spouses during the interview.

Inherent problems encountered among female spouses during the interview on sexual practices and past STDs have been reported elsewhere (26). This may also explain the less association of past STDs and HIV-infection among female spouses found in this study.

(ii) **Multiple therapeutic injections, blood transfusion and intravenous drug abuse:**

Multiple therapeutic injections at various health facilities was highly prevalent among index cases and their spouses (HIV-positive and HIV-negative). Unlike the results of a study in Zaire (74) in which an association was found between seropositivity of children of seronegative mothers and repeated medical injections, there was no association of such injections with HIV-infection of sexual partners in this study. This however needs case controlled studies to further evaluate the problem.

Blood transfusions and intravenous drug abuse were not found to be associated with HIV-infection of spouses in this study. This is unlike in western countries where during the advent of AIDS in early 1980's these risk factors were the most associated with HIV-infection (33,36,68).

(iii) **Male circumcision:**

Among sexual partners, lack of male circumcision was not found to be a co-factor for heterosexual transmission of HIV-1 in the male spouses unlike what has been reported by Plummer et al (52) in Nairobi. Uncircumcised males have been found to be at an increased risk of developing chancroid and other genital ulcers (26). These diseases in turn facilitate infection with HIV, but lack of circumcision apparently has an effect that is independent of genital ulcer disease (59). Perhaps enhanced viral survival under

the foreskin and frequent occurrence of balanitis are responsible for increased susceptibility to HIV among uncircumcised males (59).

It is possible that the small numbers of uncircumcised male spouses (23.5%) recruited in this study have contributed to lack of association between the presence of male foreskin and HIV-infection.

When the circumcised and uncircumcised male index cases were matched with their female spouses, there was no significant difference between the HIV-positive female spouses compared with their seronegative counterparts. This could be interpreted to mean that once infected with HIV-1, circumcision or lack of circumcision of males may have no role in heterosexual transmission of HIV to their female sexual partners. It may be the concentration of the HIV in the semen inoculum deposited into the female genital tract that could determine the infectivity of the HIV-infected male, rather than the presence of the foreskin in the glans penis.

Protective role of condoms:

It was not possible to evaluate the protective role of condoms adequately in the studied population due to the following reasons:

1. The target group were couples who practiced sex freely for the consummation of marriage and reproductive purposes and so most partners didn't use condoms in the past.

2. Most of the seronegative spouses on learning the HIV-seropositivity status of the index cases abstained from sex.

3. Most of the discordant couples were lost to follow up.

It is however worth pointing out that 2 male sexual partners who had used condoms regularly, one for the purpose of family planning in the past 5 years and the other to prevent HIV-1 infection with a seropositive wife have remained seronegative in the 6 months of follow up. The protective role of condoms has been shown by Ngugi et al (66) in a study conducted among Nairobi prostitutes. In the study, adequate counselling and free supply of the condoms resulted in increased frequency of their use and was associated with reduction in the risk of seroconversion for HIV-antibodies.

ABO - blood group

Studies are currently being conducted in search of intrinsic factors that are responsible for protection of some seronegative sexual partners from acquiring HIV-1 from an infected individual. Eales and colleagues (60) had started this work in Britain and currently, Plummer (personal communication) has reported HLA markers which are frequent in persistent seronegative, particular cohort of prostitutes in Nairobi. It was in view of such factors that ABO - blood groups were determined in our studied population. None of the four blood groups (A,B,AB,O) was either protective against or associated with HIV-infection of spouses when compared together. Twined to this study was determination of HLA markers for the studied population, but the results are still under evaluation.

The frequency distribution of the blood groups among spouses is closely similar to that found previously by Beecher (85) and Amolo (86) in some Kenyan tribes. The minor differences in percentage distribution of blood groups in this study compared with the other studies (85,86), may be due to the different sample sizes. In this study 118 spouses had their blood group tested. Beecher studied 5500 individuals and Amolo 1610 individuals.

Haematological and cellular immunity of index cases in relation to their spouses' HIV-infection

The primary pathogenic mechanism of depletion of CD4+T-lymphocytes in HIV infected individuals was observed in this study. Index cases, 62.5% of whom had AIDS, had a significantly depleted CD4+cell counts (mean 142/ml). Their spouses, 64.2% of whom were asymptomatic (stage 1 disease) had a mean CD4+cell count of 523/ml ($p < 0.001$). This may indicate that most of the index cases were first infected by HIV before their partners, and that they (index cases) were the primary risk for heterosexual transmission of the infection to their spouses.

Among the sexual partners, the HIV-positive spouses had significantly lower CD4+cell count (mean 433/ml) compared with their seronegative counterparts (mean 711/ml) ($p < 0.001$).

Progressive depletion of CD4+cells by HIV through various mechanisms have already been described by Fauci et al (10) and Warner (87).

Among some of the factors postulated to enhance heterosexual transmission of HIV among sexual partners is the degree of immunodepression of the infected individual. A study conducted by Van der Stuyft et al (88) in Antwerp, Belgium to determine the immunological status of HIV-infected individuals in relation to heterosexual transmission of the virus to their sexual partners,

found that index cases had a mean CD4+cell count of 341/ml which is higher than that of index cases found in this study (mean count 142/ml). The HIV-seroprevalence of sexual partners in that study (88) was 45% while the HIV-seroprevalence of spouse in this study is 70.3%. The lower CD4+cells in HIV-infected individuals found in this study compared to the Belgium study (88) may explain the higher heterosexual transmission rate of HIV in this environment.

In this study the CD4+cell count for Index Cases related to HIV-positive spouses (mean 118/ml) is lower than that of index cases related to HIV-negative spouses (mean 198/ml). Though not of statistically significant difference, ($p = 0.055$) the figures are supportive of the hypothesis that the lower the immunological status of an HIV-infected individual, the higher the transmission rate of the virus to their sexual partner.

The evidence that infectivity increases as individuals progress towards ARC and AIDS was first suggested by Goedert et al in a study of partners of men with haemophilia (55). The hypothesis that a declining immune function enhances infectivity in an HIV-infected person is biologically plausible since there is evidence that clinical deterioration is associated with enhanced virus replication (89). Thus HIV can be more readily isolated from the blood of patients with AIDS or ARC than from asymptomatic HIV-seropositive individuals (89,90). Consequently, potentially infectious body fluids such as semen and cervico-vaginal secretions

may also contain large numbers of infectious virions in these patients.

The mean white blood cell count and platelet count for index cases and their spouses were within normal ranges in this study. However, the mean haemoglobin for index cases (11.1 g/dl) was lower than that for their spouses (13.6 g/dl) Among sexual partners, the HIV-Positive spouses had a slightly lower mean haemoglobin (13.2 g/dl) compared with their seronegative counterparts (14.6 g/dl).

Anaemia is a known complication of advanced AIDS and is usually that of chronic illness as has been described by Scadden et al (25). The peripheral blood film is usually normocytic normochromic (haemoglobin levels below 11.5 g/dl) with inappropriate low reticulocyte count. The causes of anaemia include; infection particularly with mycobacterium avium complex or cytomegalovirus; tumours such as kaposis sarcoma or lymphoma and HIV-induced suppression of progenitor cells. Vitamin B_{12} malabsorption, indicated by low serum level of vitamin B_{12} levels may be due to HIV-enteropathy or tumour, is seen in upto 25% of HIV-infected patients.

The haemoglobin levels in this study though not statistically different between the groups seem to suggest that index cases had HIV infection earlier than their spouses as has already been

described in terms of CD4+cell levels of index cases compared with that of their spouses.

Clinical stage of index cases and heterosexual transmission of HIV:

In this study, no significant association was demonstrated between the clinical stage of HIV infection in the index cases and the serological status of their spouses. The same observation was reported by Padian et al (76) in the U.S.A. This is unlike the Zimbabwe study (26) in which there was increased prevalence of infection in the wives of men with ARC and AIDS. The Belgium study (88) also reported a strong association between advanced HIV-clinical stage of index cases as an independent predictor of HIV-infection of their sexual partners.

In this study and the other three studies mentioned, the HIV clinical stage of HIV infection for the index cases was obtained using the CDC clinical criteria. The discrepancy found in the various studies could be due to variable interpretations of CDC clinical staging when applied to different populations. There may also be the investigators' variability in the interpretation of the CDC clinical staging. However, the possibility that there is a high heterosexual transmission rate of HIV among asymptomatic individuals in this environment may not be ruled out.

Outcome of the studied population:

To-date the course of AIDS is invariably fatal. Of the 120 index cases, 62.5% had stage 4 disease (AIDS) at enrolment and by the end of the 6 month follow up 54.7% of them were dead. Overall 39.2% index cases died by the end of the study and a similar proportion were lost to follow up.

Of the 123 spouses 64.2%, were asymptomatic, 44.7% were lost to follow up and 1.6% were dead by the end of the study.

The high drop out rate could be due to our poor record system, inadequate infrastructure and lack of clear treatment strategies to maintain patients in the study.

Study Limitations:

1. The study was conducted among hospital based patients at the Kenyatta National Hospital in Nairobi and the results may not be generalised for the entire Kenyan population.

2. It is important to point out that the evaluated patients were only a fraction of those with suggestive signs and symptoms admitted to the medical wards. The results could not be representative of the true picture of heterosexual transmission of HIV in the entire Kenyatta National Hospital.

3. The high drop out rate especially among discordant couples could not enable the assessment of the exact number of true seronegative spouses by the end of the study period. Some of the 36 seronegative spouses could have seroconverted in the 6 months of follow up.

II - CONCLUSIONS

1. A seroprevalence of 70.7% for HIV-infection was found among spouses of HIV-infected individuals in this study.

2. Multiple sexual partners, history of genital ulcer disease and other STDs are likely co-factors involved in heterosexual transmission of HIV-1 in our environment though this did not come out very clearly.

3. Multiple injections at various health facilities though found with high prevalence among index cases and their spouses, were not associated with HIV-infection of the sexual partners in this study.

4. Lack of male circumcision was not found to be a co-factor for heterosexual transmission of HIV among spouses in this study.

5. Progressive depletion of CD4+T-lymphocytes and anemia was observed among HIV-infected individuals in the study.

6. The clinical stage of HIV-infected index cases was not found to be associated with heterosexual transmission of HIV among their sexual partners in this study.

I - RECOMMENDATIONS

1. Long term follow up of discordant couples to determine the seroconversion rate of the seronegative spouses should be carried out.

2. Further evaluation of the seronegative spouses is indicated to determine:

   Viral cultures and polymerase chain reactions for viral genome to prove true negatives.

3. A study with a well established counselling facility and free supply of condoms should be conducted to determine the protective role of condoms in our environment.

4. A case controlled study to determine the role of multiple therapeutic injections in private clinics and other health facilities should be carried. Accidental injection of medical personnel in hospitals should also be evaluated independently.

5. A study in which microbiological diagnosis for genital ulcer disease and other STDs in our environment should be carried out to determine causal association with HIV-infection.

6. It is important to maintain the essential public health message for heterosexual couples that unprotected vaginal intercourse with a seropositive person is a high risk activity for the transmission of HIV.

J - APPENDICES

APPENDIX I

Criteria for definition of adult AIDS (WHO criteria, also called Bangui criteria).

A case of AIDS in an adult is defined as a patient with no known underlying cause of cellular immunodeficiency who, presents with at least two major signs with at least one minor sign:

Major Signs:

- Weight loss of more than 10% of body weight in one month.
- Chronic diarrhoea of more than 1 month (intermittent or constant).
- Prolonged fever of more than 1 month (intermittent or constant).

Minor Signs:

- persistent cough of more than 1 month
- generalised lymphadenopathy
- herpes zoster infection
- persistent fatique
- night sweats.

APPENDIX II

TABLE

Classification of clinical stage (Centres for Disease Control) of HIV-1 infection.

Clinical Stage	Diagnosis Criteria
1	A symptomatic
2	Generalised lymphadenopathy as defined by lymphnodes in 2 non-contageous extra - inguinal sites
3	Generalised lymphadenopathy with at least two of the following symptoms present for at least one month; fever; weight loss; night sweats; pruritus; diarrhoea; chronic mucosal candidiasis maculopapular rash.
4	AIDS as defined by Centres for Disease Control or the World Health Organization

APPENDIX III

Procedure for ELISA test for HIV-antibodies by Enzygnost Anti-HIV-1/HIV-2, method by the Paul-Ehrlich - Institute-(Germany).

i) Pre-wash test plate: Remove the test plate from the container. Wash each necessary well twice with approximately 0.3ml of washing solution.

ii) Dispense sample buffer: Fill each well with a receiving volume of 50 μl of sample buffer.

iii) Dispense samples: Pipete 50 μl of negative control into 4 wells and 50 μl of the positive control into 2 wells. Dispense 50 μl of undiluted sample test into the remaining wells.

iv) Incubate: Seal the plates with adhesive foil and incubate at 37$\pm$ 1^{0}C for 30$\pm$ 2 minutes.

v) Wash: Remove the foil, aspirate all wells and wash 4 times with approximately 0.3 ml of washing solution.

vi) Dispense conjugate: Fill each well with 100 μl of working conjugate dilution

vii) Incubate: Seal with fresh foil and incubate at 37 ± 1^{0}C for 30 ± 2 minutes as described in (iv) above.

viii) Wash: Remove the foil and wash 4 times as in (V).

ix) Dispense substrate: Pipette 100 µl of washing chromogen solution into each well.

x) Incubate: Seal with fresh foil and incubate at 18^{0}C to 15^{0}C for 30 ± 2 minutes protected from light.

xi) Stop reaction: Remove the foil and add 100 µl of stopping solution peroxidase to each well keeping the same time as (x).

xii) Read at 450 nm with each hour

Sensitivity of the test for HIV-positive is 100%.

Specificity for normal negative ranges from 95.05 - 100%.

CONCLUSION

Positive tests and negative tests for HIV-antibodies will be taken as so; confirmatory tests by Western blot will not be done.

APPENDIX IV - PROFORMA

Date_____

Number_____

A. INDEX CASE

1. Name_____ _____
SEX 1: M 2 F ☐ AGE (in Yrs)

2. Present area of residence_____

Duration in area

Days	Weeks	Months	Years
☐☐	☐☐	☐☐	☐☐

3. Place of birth_____

4. Areas of regular visits if any_____

Reasons for visits_____

5. Onset of symptoms_____(date)

Approximate duration_____

6. Duration of marriage/heterosexual relationship_____

7. When did you have your last sexual intercourse with wife/husband or heterosexual partner_____

8. How many days/weeks/months/years from today had you sexual intercourse with your spouse/heterosexual partner_____

9. Possible risk factors for infection of index case.

i) About other heterosexual partners

1 - no other heterosexual contact -
2 - one other heterosexual contact
3 - two other heterosexual contacts
4 - more than 2 other heterosexual contacts

ii) Had genital ulcer or venereal disease
1- yes 2 - no

_____

when_____
treated_____where_____

iii) Had blood transfusion 1- yes 2- No

when_____

where_____

iv) Intravenous drug use (IDU) _____ 1 - Yes 2 - No

Name of drug_____

How often_____

v) Multiple therapeutic injections,

1 - Yes, 2 - No

when_____

where_____

vi) Are you circumcised? 1- yes 2 - no
(male only)

10. i) Physical examination

_____

_____

_____

ii) Clinical stage_____

iii) Treatment_____

_____

B. Sexual Partner

Date_____

Number _____

1. Name_____

Age YRS ☐☐ Sex 1 - M, 2 - F ☐

2. Area of residence_____

Duration in area_____

3. Place of birth_____

4. Areas of regular visits if any_____

_____

_____

Reason for visits_____

_____

_____

5. Post office Box_____

Telephone Number_____

work_____Home_____

Medical History Sexual Partner

1. General symptoms

1- loss of weight 5 - night sweat

2- fever 6 - pruritus

3- fatigue 7 - none of above

4 - anybody swellings

Other(s) specify _____

_____

_____

2. Systemic Inquiry

G.I.T. - Diarrhoea 1 - yes; 2- no ☐ Duration_____

- vomiting 1- yes, 2- no ☐ Duration_____

R/S - chronic cough 1- yes, 2 - no ☐ Duration_____

- chest pain 1- yes, 2- no ☐ Duration_____

C.N.S. - Headaches 1- yes, 2- no ☐ Duration_____

- weakness of any part of body 1- yes, 2- no ☐

duration_____

- Tingling sensation/paraesthesia 1- yes, 2- no ☐

Duration_____

- Numbness 1- yes, 2- no duration_____ ☐

C.V.S. - Palpitation 1- yes 2 - no ☐ Duration_____

1-Yes, 2-No

Skin - any new or old rash _____ ☐

Duration _____

Other(s) specify _____

_____

_____

SEXUAL HISTORY

1. Sexual practices and protective or risk factors

i) Relationship with index case

1- spouse (husband/wife) ☐

2 - heterosexual partner (friend, workmate, others (specify)

_____

_____

_____

ii) Duration of marriage/heterosexual relationship with index case _____

iii) Other co-wives (for women only)

1- only wife

2- 1st wife of _____ other(s) ☐

3- 2nd wife of _____ other(s)

4- 3rd wife of _____ others

5- 4th wife of _____ others

6- 5th wife of _____ others

iv) When did symptoms of your husband/wife or heterosexual partner's illness begin?

- Date_____

Duration_____

v) When did you last have sexual intercourse with your husband/wife/sexual partner

Approximate date_____

Duration_____

vi) Approximately how often do you have sexual relationships with your husband/wife/heterosexual partner

1 - never
2- less than twice a month
3- once a week
4- twice a week
5- more than twice a week

vii) Since onset of symptoms of your wife/husband/heterosexual partner, has sexual practice changed?

1- continued having sex_____

2- abstained from sex_____

viii) Use of condoms

1- Don't use condoms

2- occasionally use condoms

3- always use condoms

Reasons for use_____

2. Other possible risk factors of infection to the spouse/heterosexual partner

i) about other heterosexual partners

a) number

1- no other heterosexual partner
2- one other heterosexual partner
3- two other heterosexual partners ☐

4- more than two other heterosexual partners

b) When did you last have sexual intercourse with this or these other heterosexual partners?

- approximate date(s) _____
_____
_____

- duration _____
_____
_____

c) How often did you have sexual intercourse with this or these other heterosexual partner(s)

_____

_____

_____

ii) Blood transfusion 1- yes 2- no ☐

when _____

where _____

iii) Genital ulcers or venereal disease

1- yes 2- no ☐

when _____

treated _____ where _____

iv) Are you circumcised?

(Males only)
1- yes 2- no ☐

when_____

where_____(Home/Hospital)

v) Intravenous drug use (IDU) 1- yes 2- no

☐

Name of drug_____

How often is it used_____

vi) Multiple therapeutic or accidental injections

1- yes 2- no

☐

when_____

where_____

PHYSICAL EXAMINATION

1. General

Fever_____Temperature_____

Pallor_____

Lymphadenopathy_____

Wasting_____

Oedema_____

Other(s) (Specify)_____

2. Systems:-

Skin_____

_____

G.I.T._____

_____

R/S_____

_____

C.V.S._____

_____

C.N.S._____

_____

Other(s) specify_____

_____

_____

3. i) Clinical diagnosis at 1st Visit

_____

_____

ii) ELISA result for HIV- ab at 1st visit

1- positive; 2- negative ☐

iii) Haemogram results

Hb_____WBc_____

PLT_____

Other indices_____

_____

iv) Blood group_____

v) T - lymphocyte counts

CD4+_____

CD8+_____

CD4+; CD8+ Ratio_____

Treatment_____

vi) Other investigations (specify)_____

_____

_____

FOLLOW UP

. Second visit - 2 months follow up

Date_____

. Index case:

Physical examination_____

Clinical diagnosis_____

Treatment_____

. Sexual partner

Date_____

i) Any complaints_____

ii) Sexual practices with index case

1- regularly unprotected sex ☐

2- occasionally unprotected sex
3- always use condoms
4- abstained from sex

iii) Physical examination_____

iv) Clinical diagnosis at 2 months follow up

Treatment_____

v) ELISA report at 2 month follow up 1- positive, 2- negative ☐

vi) Haemogram results: Hb_____

PLT_____

WBC_____

5. 3rd visit - 4 months follow up

Date_____

A. Index case:

Physical exam_____

Clinical diagnosis_____

Treatment_____

B. Sexual partner

Date_____

i) Any complaints_____

ii) Sexual practices for seronegative spouse

1- regularly unprotected sex
2- occasionally unprotected sex
3- always use condoms
4- abstained from sex ☐

iii) Physical Examination

_____

iv) Clinical diagnosis at 4 months follow up

_____

Treatment_____

v) ELISA result for HIV - ab 1- positive 2- negative

☐

vi) Haemogram results: Hb_____ WBC_____
PLT_____

viii) Other investigations (specify)

_____

_____

6. 4th Visit - 6 months follow up
A. Index Case: Date_____

Physical examination_____

Clinical diagnosis_____

Treatment_____

B. Sexual partner: Date_____

i) Any complaints_____

_____

ii) Sexual practices with index case -

1- regularly unprotected sex
2- occasional unprotected sex ☐

3- always use condoms
4- abstained from sex

iii) Physical examination_____

_____

iv) Clinical diagnosis at 6 month follow up

_____

Treatment_____

v) ELISA report for HIV- ab at 6/12
follow up 1- positive 2- negative

☐

vi) Haemogram results Hb----------
WBC_____
PLT_____

vii) Other investigation (specify)

_____

_____

_____

J - REFERENCES

1. Ratner L., Gallo R., Wong-Staal F.
HTLV-III, LAV, ARV are all variants of the same AIDS Virus.
Nature 1985; 313: 636-637.

2. Anthony S., Gallo R., Scott K., Salk J. et al.
Development and evaluation of a vaccine for HIV-infection.
Ann. Intern. Med. 1989; 110; 373-385.

3. Siegal F., Lopeze C., Hammer G. et al.
Severe acquired immunodeficiency in male homosexuals manifested by chronic peri-anal ulcerative herpes simplex lesions.
N. Engl. J. Med. 1981; 305: 1439-1444.

4. Barre-Sinoussi F., Cherman J., Rey F., et al.
Isolation of T-lymphotropic retrovirus from a patient at risk for AIDS.
Science 1983; 220: 868-871.

5. Gallo R., Salahuddin S., Popoic M., et al.
Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS.
Science 1984; 224: 500-503.

6. Clarel F., Guetard F., Brun-vezinet S. et al.
Isolation of a new retrovirus from West African patients with AIDS.
Science 1986; 233; 343: 343-346.

7. Bavin F., M'Boups, Denis F. et al.
Serological evidence for virus related to Simian T-lymphotropic retrovirus III (HTLV-III) in residents of West Africa.
Lancet 1987; 1: 388-390

8. Wede N.
Immunologic Considerations of Paediatric HIV-infection.
J. of Paed. 1981; 119: 55-57.

9. Fauci A.
The human immunodeficiency virus; infectivity and mechanisms of pathogenesis.
Science 1988; 239: 617-622.

10. Fauci A., Schnittman S., Guido P., et al.
Immunologic mechanisms in human immunodeficiency virus (HIV) infection.
Ann. Intern.Med. 1991; 114: 678-693.

11. Piot P., Kreiss J., Ndinya-Achola J.,
Heterosexual transmission of HIV.
AIDS: 1987; 1: 199 - 206.

12. Ammann A., Schiffman G., Abrams D. et al
B-cell immunodeficiency in acquired immunodeficiency syndrome (AIDS)
J.A.M.A. 1984; 251: 1447-1449.

13. Lane H., Masur H., Edgar L. et al
Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome.
N. Engl. J. Med. 1983; 309: 453-458.

14. Fischl A., Gordon M., Scott B. et al
Evaluation of Heterosexual Partners, Children and Household contacts of adults with AIDS.
J.A.M.A. 1987; 257:642 - 644.

15. Girran J.; Jaffe W.; Hardy A. et al
Epidemiology of HIV-infection and AIDS in the U.S.A.
Science 1988; 239: 610-615.

16. Ansary M., Hira S., Bayley A. et al
A colour Atlas of AIDS in the Tropics
Wolfe Medical Publications Ltd. 1989; pg 14-16.

17. Yarchoan R., Pluda J.
Aetiology, Diagnosis, treatment and prevention.
AIDS, Textbook, second edition copyright J.B. Lippincort company 1988 pg 107-117.

18. Murray N., Mills J.
Pulmonary Infectious Complications of Human Immunodeficiency virus infection.
Am. Rev. Resp. Dis. 1990; 141: 1356-1369.

19. Cohen H., Donovitch G., Parsa P. et al.
Human immunodeficiency virus (HIV) infection and the Kidney.
Ann. Intern. Med. 1990; 112: 35-47.

20. Quinn T., Strober W., Janoff E. et al.
Gastrointestinal Infections in AIDS
Ann. Intern. Med. 1992; 116: 63-74.

21. Kaye R.
Rheumatological manifestations of infection with human immunodeficiency virus (HIV).
Ann. Intern. Med. 1989; 111: 158-167

22. Elder A., Sever L.
Neurologic Disorders associated with AIDS retrovirus infection.
Rev. Infect. Dis. 1988; 10: 286-295.

23. Tania J., Jeffrey S.
Recent Advances on Dermatology
N. Engl. J. Med. 1992; 326: 167-176.

24. Tappero W., Conant A., Wolfe F. et al
Kaposis Sarcoma: Epidemiology, histology, clinical spectrum, staging criterior and therapy.
J. Am. Acad. Dermato. 1993; 28: 371-387.

25. Scadden T., Zon I., Groopman E.
Pathophysiology and management of HIV-associated Haematologic Disorders.
J. Am. Soc. Haem. 1989; 74: 1455-1460

26. Marowa E., Stan H., Latif S. et al
Genital Ulcers and transmission of HIV among couples in Zimbabwe.
AIDS. 1989; 3: 519 - 523.

27. World Health Organisation (WHO)
World distribution of HIV/AIDS and Global Epidemiology patterns.
WHO/GPA/DIR/ 1990; 4: 2-5.

28. World Health Organisation (WHO)
Current and Future Dimensions of the HIV/AIDS pandemic.
WHO/GPA/RES/SFI/ 1991; 4: 2-12.

29. World Health Organisation (WHO)
Weekly Epidemiological records for Acquired Immunodeficiency Syndrome as of 30th June 1993.
1993; 68: 193-196.

30. Gottlieb M., Schroff R., Schranker H. et al
Pneumocystic Carinii Pneumonia and mucosal Candidiasis in previously health homosexual men. Evidence of new acquired immunodeficiency.
N. Engl. J. Med. 1981; 305: 1425-1431

31. Curran J., Lawrence D., Jaffee H. et al
Acquired immunodeficiency Syndrome (AIDS) as associated with transfusion.
N. Engl. J. Med. 1984; 310: 69-75.

32. Oleske J., Minner A., Cooper R. et al
Immunodeficiency Syndrome in children.
J.A.M.A. 1983; 249: 2345-2349.

33. Goedert J., Bigger R., Sarngadharan M. et al
Determinants of retrovirus HTLV-III antibody and immunodeficiency conditions in homosexual men.
Lancet 1984: 2: 711-715.

34. Kingsley L., Detels R., Kaslow R. et al
Risk factors for seroconversion to human immunodeficiency virus among male homosexuals: Results from the multicentre.
AIDS Cohort Study.
Lancet 1987; 1: 345-348.

35. Marmor M., Weiss L., Lyden M. et al .
Possible female transmission of human immunodeficiency virus.
Ann. Intern. Med. 1986; 105: 969.

36. Morizon O., Capellan J.
Female-to-female transmission of HIV.
Lancet 1987; 2: 40-41.

37. Chiason R., Moss A., Onishi L. et al.
Human immunodeficiency virus infection among heterosexual intravenous drug users in Sanfransisco.
Am. J. Pub. Health. 1987; 125: 1035.

38. Wofsy C., Cohen J., Hauer L. et al.
Isolation of AIDS associated retrovirus from genital secretions of women at risk for AIDS.
Lancet 1986; 1: 527-529.

39. Vogt M., Witt D., Craven D. et al
Isolation of HTLV-III/LAV from cervical secretions of women at risk for AIDS.
Lancet 1986; 1: 525- 526.

40. Harris C., Small C., Klein R. et al.
Immunodeficiency in female partners of men with the acquired immunodeficiency syndrome.
N. Engl. J. Med. 1983; 308: 1181-1184.

41. Kreiss J., Kitchen L., Kasper C. et al.
Antibody to HTLV-III in wives of haemophiliacs. Evidence of heterosexual transmission.
Ann. Intern. Med. 1985; 102: 623-626.

42. Van De Perrep P., Clumlek N., Carael M. et al.
Female prostitutes a risk group for infection with HTLV-III.
Lancet 1985; 2: 524-526.

43. Melbye M., Njelesani E. Bayley A. et al.
Evidence of heterosexual transmission and related conditions in Lusaka, Zambia.
Lancet 1986; 2:1113-1115.

44. Poit P., Yaelman H., Minlangu K. et al.
Acquired immunodeficiency syndrome in heterosexual population in Zaire. Lancet 1984; 2:65-69.

45. Kreiss J., Koech D., Plummer F. et al.
AIDS Virus infection in Nairobi prostitutes: Spread of the epidemic to East Africa.
N. Engl. J. Med 1986; 314;414.

46. Pepin J., Plummer F., Brunham R. et al.
The interaction of HIV-infection and other sexually transmitted diseases: an opportunity for intervention
AIDS. 1989; 3: 3-8

47. Metroka C., Cunningham R., Pollack M. et al.
Generalised lymphadenopathy in homosexual men.
Ann. Intern. Med. 1983; 99: 585-591.

48. Piot P., Holmes K.
Sexually transmitted diseases.
Tropical and Geographical Medicine Edited by Warren K S et al.
New York McGraw-Hill; 1984: pg 844-862.

49. Hardy D.
Cultural Practices contributing to the transmission of HIV in Africa.
Rev. Infect. Dis. 1987; 9:1109-1119.

50. Kreiss J., Coombs R., Plummer F. et al.
Isolation of Human Immunodeficiency Virus from genital ulcers in Nairobi prostitutes.
J. Infect. Dis. 1989; 160:380-384.

51. Jessamine P., Plummer F., Ndinya-Achola J. et al.
Human immunodeficiency virus, genital ulcers and male foreskin: Synergism in HIV-1 transmission.
Scand. J. Infect. Dis. Suppl. 1990:181-186.

52. Plummer F., Simonsen N., Ngugi E. et al.
Incidence of human immunodeficiency virus (HIV) infection and related diseases in a cohort of Nairobi prostitutes.
Abstract M.8.4; third International Conference on AIDS Washington DC June 1-5, 1987.

84. Piot P. Plummer F., Mhalu F. et al
AIDS: An International Perspective.
Science 1988; 264: 2599 - 2602.

85. Beacher J.
ABO blood group distribution in some Kenya tribes.
E. Afri. Med. J. 1967; 44: 134 - 138.

86. Amolo M., Chiniyara A.
Blood group distribution in West Kenya populations
E. Afri. Med. J. 1984; 61: 208 - 301.

87. Warner C.
Molecular Biology of HIV - 1
N. Engl. J. Med. 1991; 324: 308 - 315.

88. Van der Stuyft P., Laga M. Taelman H. et al
Advanced Immunodeficiency as a risk factor for heterosexual transmission of HIV.
AIDS (J) 1989; 3: 361 - 366.

89. Lange J., Paul D., Huisman G. et al
Persistent HIV antigenaemia and decline of HIV core antibodies associated with transmission of AIDS.
Br. Med. J. 1986; 293: 1459 - 1462.