PREVALENCE OF ANTI-HIV ANTIBODIES IN PROSTITUTES AND THEIR CLIENTS IN ADDIS ABABA

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ABSTRACT

Acquired Immunodeficiency Syndrome (AIDS), caused by Human Immunodeficiency Virus (HIV), is becoming a world challenge and concern. To determine the prevalence of anti-HIV antibodies among groups of high risk behaviour in Addis Ababa, 494 subjects (mean age 27.9, range 12-75 years) were studied. The study group comprised 177 prostitutes (153 from Addis Ababa, 24 from Chencha), 140 male clients (130 from Addis Ababa, 10 from the countryside), and 177 controls both from Addis Ababa and the rural areas. Out of the Addis Ababa group 9 (5.8%) of the prostitutes and 4 (3%) of the male clients were found to be seropositive by both ELISA and Western blot tests. In the seropositive prostitutes the peak prevalence (55.5%) occured in those aged 15-24 years and in seropositive male clients (75%) occurred in the 25-34 years age group. The infection rate between female prostitutes and male clients was found to be 2.5 to 1. With the exception of one male client, prevalence of antibody was not associated with clinical symptoms.

This limited sero-epidemiological study has determined seropositive individuals within the Addis Ababa region. The low number of seropositive individuals in these high risk behaviour groups, when compared with reports from other African countries, may suggest a more recent establishment of HIV infection in Addis Ababa.

INTRODUCTION:

In June 1981, the United States Centers for Disease Control (CDC) reported an unusual outbreak of deaths from opportunistic infections such as Pneumocystis carinii pneumonia (PCP) or neoplasm such as an aggressive type of Kaposi's sarcoma (KS) (Gottleib, et al. 1981; Masur, et al., 1981, and Siegal, et al., 1981). The deaths were largely confined to previusly healthy homosexual men. These two diseases were well studied and were not been known to progress as fast as in these mysterious case. Immunological investigations in these patients indicated that they were immunosuppressed and hence the new syndrome was called Acquired Immunodeficiency Syndrome (AIDS). According to the CDC, AIDS is defined as: "A reliably diagnosed opportunistic infection that is moderately indicative of an underlying cellular immune deficiency or neoplasm such as Kaposi's Sarcoma in previously healthy individuals" (Inderlied and Young, 1985). However, the clinical definition of the World Health Organization (WHO, 1985), elaborates further the major and minor symptoms of the disease. According to this definition, the major signs of AIDS are weight loss ($\geq$10 percent body weight), chronic diarrhaea which persist for more than one month, and prolonged fever which may be intermittent or constant. The minor signs include persistent cough for more than one month, generalized pruritic dermatitis, recurrent herpes zoster, oropharyngeal candidiasis, chronic progressive and disseminated herpes simplex infection, and generalised lymphadenopathy. According to a WHO memorandum AIDS is recognized by the existance of at least two major signs associated with one minor symptom. But when patients show two of the minor and one or more the major signs the condition is

known as AIDS Related Complex (ARC). However, in Africa where there is lack of adequate laboratory facilities, malnutrition and other life threatening illness have similar symptoms, to AIDS and consequently one needs maximum care and a pilot study, to evaluate the clinical picture of AIDS patients. AIDS is usually a fatal disease and upto 80% of the AIDS patients in the USA are expected to die within 3 years time (Population Reports, 1986). Up to now no efficacious treatment has been found and the hope of producing a vaccine seems a thing for the future.

Initial explanations for the cause of AIDS included several hypothetical factors such as antigen overload, the effects of chronic amyl nitrite use, and the immunosuppressive properties of sperm (Shearer and Robson, 1984). However, on the basis of the increasing incidence of AIDS, the type of social groups affected, and seroepidemiological data, the suggestion that an infectious agent, possibly a virus, was responsible for the transmission of the disease was put forth (Essex, et al., 1983). In addition, laboratory investigations showed that AIDS was associated with the depletion of helper/inducer T-lymphocytes (T4) (Koziner, et al., 1982; Gold, et al., 1982). The observation of immunosuppression and susceptability to opportunistic infections in cats following infection by Feline Leukemia virus (FeLV) - a retrovirus (Trainin, et al., 1983) gave an additional clue for the suggestion that a virus, possibly a retrovirus could be the causative agent.

In 1983 and 1984 researchers working independently in France and the United States of America discovered a novel human retrovirus from lymphadenopathy and AIDS patients respectively (Barre-Sinoussi, et

al., 1983; Gallo, et al., 1984). Unlike the two previously isolated human Leukemia viruses, Human T-cell Leukemia Virus type I (HTLV-I) (Poiszy, et al., 1980) and Human T-cell Leukemia Virus type II, (HTLV-II) (Kalyanaraman, et al., 1982), this new virus was not oncogenic but was cytopathic and caused immunosuppression. The virus was initially isolated from a swollen lymph node and was designated as Lymphadenopathy Associated virus (LAV) by the French group and the virus isolated from AIDS patients by the American group was named as Human T-cell Lymphotropic Virus Type III (HTLV-III). This virus is responsible for the new disease AIDS.

Once the AIDS virus is inside the human host, specific antibodies are produced within 2 to 24 weeks (Anony, 1984; Montagnier, et al., 1985) and progressive ill health usually follows between six and sixty months (WHO 1985). Hence, in almost all cases antibody production is an indicator of LAV/HTLV-III infection. Furthermore, infected individuals are occasionally virus positive, but symptom free and seronegative (Salahuddin, et al. 1984, Groopman, et al., 1985). The presence of anti-AIDS virus antibody has been detected by a variety of serological techniques including Enzyme Linked Immunosorbent Assay (ELISA) and Western blot. Since a viral antigen assay has not yet been reported, viral exposure can only be detected by anti LAV/HTLV-III antibody assay and/or virus isolation.

Since the AIDS virus has a long period of latent infection (WHO, 1985) it is difficult to tell how many healthy carriers will succumb to AIDS or ARC. According to one estimate (WHO, 1986), from the healthy carriers 10% will develop AIDS, 20-30% will develop ARC and 40-70% remain asymptomatic carriers. It is not known whether in the future

this huge number of asymptomatic carriers will develop clinical signs of AIDS.

AETHOLOY OF AIDS

The virus that causes AIDS is most commonly referred to as LAV/HTLV-III although the International Committee for the Taxonomy of Viruses has recently proposed the name Human Immunodeficiency Virus (HIV) (Cofin, et al., 1986; and Marx 1986), which will be used in this dissertation. Recently other related retroviruses that cause AIDS have been isolated from AIDS patients and were grouped under the HIV family and were designated as HIV II and HIV III while the first virus was named as HIV I.

HIV is a member of the Retroviridae family. Retroviruses are RNA viruses (the RNA serves as a template) and information flow is from RNA to DNA and then back to RNA and finally to proteins (Gluckman, et al., 1986; Gallo, 1986). These viruses have a unique enzyme called reverse transcriptase (RT) that can transcribe DNA copies of the RNA viral genome. This process is a reversal of the normal information flow and hence the name retrovirus. Many retroviruses are known to cause disease in higher vertebrates (Gallo, 1985; Salahuddin, et al., 1985). Retroviruses were also known to cause cancer in animals and hence they were once named as the RNA tumor viruses (Luria, et al., 1978; Joklik, 1980; Broder and Gallo, 1985). However, many retroviruses cause non-malignant disease and non-pathogenic conditions. One basic feature shared in common by all retrovirus infections is that infected individuals remain infected, often asymptomatically, for prolonged periods, probably, for life (Gonda, 1986).

CLASSIFICATION OF HIV

After classifying HIV in the family Retroviridae, scientists still differ as to which sub-family HIV belongs. According to Gallo et al., (1984) HIV should be placed in the human T lymphotrophic virus (HTLV) group, because the virus shows some common properties with HTLV-I and -II such as T-cell tropism, possession of reverse transcriptase, induction of multi-nucleated giant T-cells, possession of cross reacting antigens, and immunosuppression activity in vitro and in vivo.

However, according to Montagier (1985); Alizon et al. (1985) and Kan, et al. (1986) although HTLV-I and HIV have similarities in that they are both retroviruses that have CD4 tropism, their genes are distinct from HTLV-I and HIV has many similarities with lentiviruses which cause degenerative neurological disease in sheep. The similarities of HIV and lentiviruses include cytopathic effect in vitro, unintegrated DNA in infected cells, neurotropism, and the presence of large glycoproteins.

Although the classification is unsettled the presence of four extra genes (tat, trs, sor and 3′ orf) (Gallo, 1987) and the separation of the pol and the env genes, which overlap in HTLV-I, makes it probable that HTLV-I and HIV belong to different sub-families.

MODE OF TRANSMISSION HIV INFECTION

To date, HIV has been isolated form blood, semen, saliva, tears, breat milk, vaginal/cervical secretions, urine, cerebrospinal fluid and brain tissues (Zagury, et al., 1984; Ho, et al., 1984; Groopman, et al., 1984; Fujikaw, et al., 1985; Ho, et al., 1985; Thiry, et al.,

1985; Levy, et al., 1985; Wofsy, et al., 1986; Vogt, et. al., 1986; Koening, et. al., 1986). Among these blood and semen appear to be the most important in the transmission of the virus in humans.

Thus, the modes of transmission include: sexual contact, direct contact with contaminated blood and blood products, transfer of the virus via breast milk, or across and the placenta from infected mothers to their offspring (Curran et al., 1985; Pitchenic, et al., 1984). On the other hand the transmission of HIV through less intimate means such as kissing, touch, breathing, sharing of eating and drinking utensils. sharing of showers and towels and the like is controversial. Even after prolonged close contact, (22-37 months) family members and friends of AIDS patients did not contract AIDS or developed anti-HIV antibodies (Saltzman, et al., 1986; Peterman, et al., 1986). There is also no evidence for arthropod transmission of AIDS (Piot and Schofield, 1986) as yet, but such vectors may be possible disease transmitters.

ORIGIN OF HIV VIRUS

The origin of HIV I is, like its nomenclature and classification, is controversial. At present, there are several hypothesis on the origin of the virus.

1. HTLV-I, which originates in Africa, could be the ancestral origin of HIV (Gallo, 1985; Brun-Vesinet, F., 1985).

   Gallo (1985) and Essex (1985) have suggested that HTLV-I, (the ancestral origin of HIV) was brought to America by the slave trade and to the Southern part of Japan before the sixteen century with the seamen from America. However, it is

probable that HTLV-I was endemic in Japan long before the advent of the slave trade (Ishida, 1986).

2. The virus has always been a human pathogen but has been restricted to a small number of people (Desrosiers, 1986).

The rapid spread of AIDS in urban areas is similar to the epidemic of Visna Virus in sheep in Iceland following the import of infected animals from West Germany (killing about 150,000 Icelandic sheep between 1939-1952) (Gonda, 1986). Historically, there are many cases where a pathogen has stayed latent for a long period of time in an endemic area where the bulk of the community is immune, and at other times, for unknown reasons, the same causative agent can spread beyond its natural focus. As a result of extensive political, economical, diplomatic and social interactions among countries there have been increased opportunities for the migration of pathogens.

3. The progenator of HIV virus may be an ancestral human or animal virus which subsequently changed its pathogenicity and/or host (Clumeck, 1985; Desrosiers, 1986a).

Bovine leukemia virus (BLV) will cause T-cell leukemia when introduced to sheep and an AIDS like disease when inoculated into rabbits (Gallo, 1985). Just the same, no direct evidence is available at the for HIV to support this hypothesis.

Viruses similar to HIV have been found in monkeys. The first " HIV like" virus was isolated from Asia in wild caught primates, Macacca mulatta, and was named Semian T-lymphotropic virus type III (STLV-III MAC) (Daniel, et al., 1985). In addition, antibodies to STLV-III MAC have been detected in wild African green monkeys, Cercopithicus aethiops, (Kanki, et al., 1985). Soon after this observation Kanki (1985) was able to isolate an "STLV-III like" virus which was designated as STLV-III AGM. She also mentioned that STLV-III Mac and STLV-III AGM cause immunodeficiency among macaques and asymptomatic infections in African green monkeys respectively. In addition to these monkey viruses, Clavel , et al., (1986) isolated another type of human retrovirus from AIDS patients in Guinea Bissaw and the Cape Verde Islands which was initially named as LAV-2 and renamed as HIV-II (Clavel, et al., 1986). A fourth type of human retrovirus was isolated from healthy carriers in Senegal during large scale screening of populations for the prevalence of anti-HIV antibodies. (Kanki, et al., 1986). All these findings led Kanki, et. al. (1985); and Clavel, et al., 1986; and Newmark, 1986) to hypothesize that an apparently harmless monkey virus, STLV-III AGM was the origin of a harmless human retrovirus, HTLV-IV which gave rise to a pathogenic HIV-II, the ancestor of HIV-I.

More investigation is required to draw a phylogenetic tree to the HIV virus, since 8 months after the first isolation of the so called

"harmless human retrouirus", HTLV-IV, a Guinea Bissaw woman, with no anti-HIV antibodies, but with a chronic fatal illness resembling AIDS was found to be seropositive for anti-HTLV-IV antibodies (Molbak, et al., 1986). Another HTLV-IV seropositive case, with no anti-HIVI antibodies, but with clinical symptoms was reported from a Gambian woman living in Sweden (Biberfeld, et al., 1986). It is also true that except HIV-I both HIV-II and HTLV-IV are not sequenced and detailed study may indicate the fall of HIV-II and HTLV-IV into the same species (Clavel et al., 1986).

LIFE CYCLE OF HIV

The first event in the life cycle of retroviruses is the binding of the virus envelope glycoprotein with the receptor for the virus located on the membrane of the host cell. In the case of HIV the CD4 (T4) molecule or a similar molecule acts as a receptor for the virus (Klatzmann et al., 1984). Consequently those cells which have the CD4 molecule or a similar molecule are possible target of HIV infection. Although the CD4 lymphocytes are the principal target cells (Barnes, 1986) some other cells like the monocytes/macrophages, some B cells, brain astrocytes and microglial cells can also be infected (Ho et al., 1985; Barnes, 1986) because of the possesion of CD4 receptos or a similar molecule. Soon after the fusion of the virus envelope protein with the host cell membrane, the virus core protein is taken into the host cell by endocytosis, leaving behind its envelope protein on the surface of the host cell membrane. Once the HIV core protein is inside the cytoplasm it sheds its coat and the enzyme reverse transcriptase becomes active and transcribes viral RNA into DNA . The

single strand DNA copy becomes double stranded and becomes circularized, finally migrating to the host nucleus and integrating with the host cell chromosome forming a provirus (Gallo, 1986). This first phase is similar in all retroviruses (Luria, et al., 1978; Griffinth et al., 1986). The proviral HIV DNA may remain silent (unexpressed state) for a long period of time (Norman, 1985 and Griffith, 1986) or it may express itself using host cell mechanisms following antigenic stimulation. When expressed, the integrated viral DNA is transcribed into RNA, which is then translated into structural proteins and enzymes which are assembled at the host cell membrane to form complete virions which are released from the cell by budding.

PATHOGENICITY OF HIV

Unlike the leukemia viruses which bring about uncontrolled growth of host cells, expression of the previous HIV leads not only to the death of the infected cells but also to the spread of the virus to new susceptable cells (Selwyn, 1986). However, the mechanism through which HIV induces cytopathogenic effect *in vivo* is not clearly known. But there is evidence a from *in vitro* studies as to how HIV might kill its target cell.

(1) Formation of multinucleated giant cells or syncytia (Resnick, *et al.*, 1986; and Klatzmann, *et al.*, 1986)

The presence of the viral glycoprotein on the surface of the infected cells, can bring about the fusion of infected and non-infected CD4 positive cells. This fusion will result in the formation of multinucleated giant cells also known as syncytia. This giant cell formation leads to cell death within 24 hours after their formaiton, although the mechanism is poorly understood (Montagnier, *et al.*, 1985; Gluckmann, *et al.*, 1986). According to Lifson (1986) and Resnick, *et al.*, (1986), infected cells displaying viral envelope glycoprotein on their surface can fuse with and kill non-infected CD4 positive cells .

(2) Cytotoxic killing of the CD4 cells (Wain-Hobson and Montagnier, (1986).

The CD4 cells are killed by a CD8 cytotoxic subset of T-cells as a result of the infected CD4 lymphocytes displaying

HIV antigens on their surface are considered by the cytotoxic T-cells as foreign invaders (Singer, et al., 1986).

(3) Release of soluble factors.

Dying HIV infected cells releases a soluble factor or protein which incapacitates nearby CD4 lymphocytes, possibly by disrupting the receptor mechanissm of the CD4 lymphocytes and consequently the CD4 cell cannot recognized antigen (Laurence, 1985).

(4) Formation of holes on the lymphocyte membrane when the virions are liberated. When the virus reproduces itself, the virions liberated from the lymphocytes make holes in the lymphocytic membrane which might lead into cell death (Gallo, 1986). The reason given by Gallo was that as very large numbers of virus bud in a mass of particles the cell cannot repair the holes immediately and as a result there will be a failure to maintain the proper intracellular ionic environment which finally brings the death of the infected cell.

Whatever the mechanism of CD4 killing may be, after a certain critical point this process brings about the depletion of a significant proportion of the CD4 cells. The CD4 to CD8 ratio, in infected individuals with clinical symptom is usually below 0.9 compared to 2:1 in normal individuals. This CD4 cell depletion will make the individual vulnerable to opportunistic infections.

HIV INFECTION

HIV infection takes many forms before it reaches its end stage of clinical disease, AIDS (Fig. 1). Some of these stages are summerized as follows (Population Reports, 1986).

(1) Development of anti-HIV antibodies (seroconversion). In most cases HIV infection stimulates the production of antibodies. So far, serological studies have indicated the presence of HIV infection in more than 112 countries (TABLE 1).

(2) Asymptomatic carriers state

Most infected individuals are asymptomatic healthy carriers (Fig. 1) and can only be detected by serological techniques. However, these asymptomatic carriers can transmit the virus and hence are potential source of infection (May and Anderson, 1987).

(3) Development of AIDS related compex (ARC)

Individuals with ARC show some clinical symptoms but do not necessarily progress to the fatal form of the disease. ARC may be a transitional stage between the symptom free, seropositive individuals, and full blown AIDS cases. This state is manifested by opportunistic infections and neoplasmic diseases such as Kaposi's sarcoma which arise as a result of depressed immunity. Moreover it is also possible that antibody positive healthy carriers can develop clinical AIDS without entering into the transition stage.

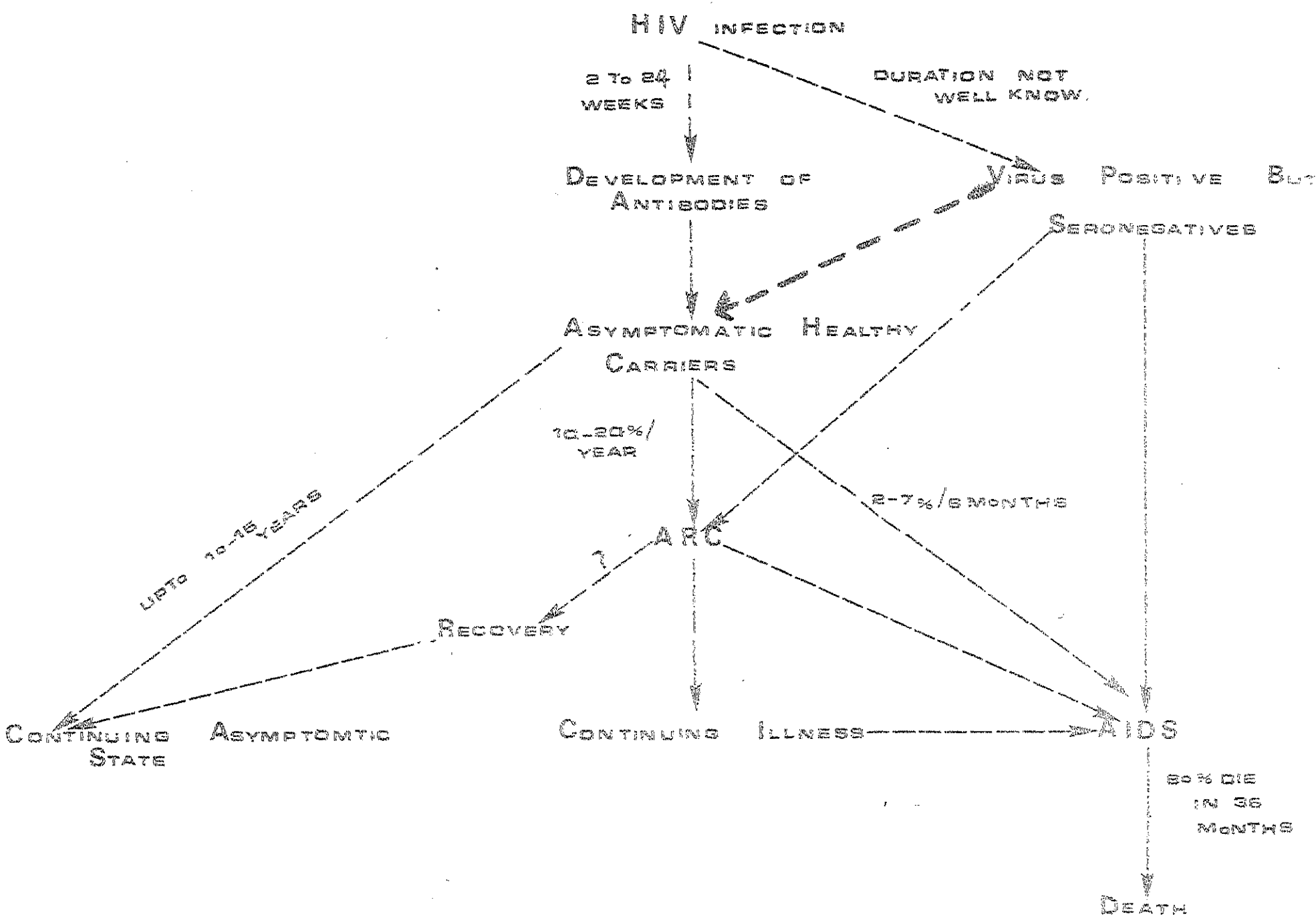


FIG.1. SPECTRUM OF HIV INFECTION

(4) AIDS

This is the final stage of the diseased characterized by either repeated life threatening opportunistic infections or skin tumors such as Kaposi's sarcoma.

However, cofactors that may promote the development of the clinical signs of HIV infeciton are poorly understood although it is possible that repeated exposure to foreign antigens may be responsible for the induction of HIV replication (Selwyn, 1986).

HOW DOES HIV ESCAPE THE HOST IMMUNE RESPONSE?

Some of the possible mechanisms that enable HIV to escape the host immune response are:

(1) By killing cells of the immune system. CD4 cells which are essential to the induction of a normal immune response are directly killed by the virus. This suppresses the immune response to HIV and other antigens (Gallo, 1987).

(2) By forming giant cells

The formation of syncytia, giant cells, may facilitate the passage of virions intracellularly which in turn avoids inactivation by circulating neutralizing antibodies, in other words the virus travels from one cell to the next without being exposed to the immune system (Resnick, et al., 1986). Although the syncytia dies within 24 hrs. this time may be enough for intensive viral replication.

(3) Using the brain as a reservoir of virus

HIV escapes the host's immune response by hiding itself in an immunologically previlaged site like the brain. Hence, the brain could act as a reservoir of the virus. The virus' neurotropism in some advanced stage patients may be an example of this.

(4) Genetic variability (Alizon et al., 1986)

Antigenic drift and shift may serve as one means for the virus to escape the host's immune response (Essex et al., 1985; Haase, 1986). HIV shows high degree of heterogenity. One isolate is different from another isolate, particularly with respect to the envelope glycoprotein antigen (Hahn, et al., 1985). The rate and degree of genomic variation in HIV is very high when compared with other retroviruses (Essex, 1985). This variability may be due to errors made by the enzyme reverse transcriptase during transcription (Coffin, 1986). Hence the antibody produced against one strain of HIV may not be protective for another type of strain. This might be one of the reasons why HIV escapes the host's immune response.

EPIDEMIOLOGY OF AIDS

Although AIDS has been recognized only 8 years ago over 50,000 people in more than 100 countries are affected (WHO, 1987) of whom more than 36,000 are from the Unites States of America. In Europe the number is lower (5,100) which is similar to Africa (See Table -I).

TABLE I
AIDS CASES IN 5 CONTINENTS as reported by
WHO, May 1987

Continent	No. of Cases	No. of Reporting countries
Americas	40,500	33
Europe	5,100	27
Oceania	404	2
Africa	5,000	27
Asia	103	15
TOTAL	50,703	104

The number of AIDS cases is almost certainly under reported and may exceed 100,000 (WHO, 1987) since the reported cases of AIDS most probably represent only the tip of the iceberg with many cases unrecognized. In addition to AIDS, 300-500 thousand people have ARC and 5-10 million people might be asymptomatic carriers capable of transmitting HIV (WHO, 1987).

The global prevalence of AIDS is increasing rapidly. If we consider the US, where intensive AIDS research and case monitoring is going on, the case doubling time of AIDS was 5 months in 1981-82, 8 months in 1983-84, and in 1985 to mid 1986 it slowed down to 11-12 months (Selwyn, 1986). This decline in the case doubling time may be

accounted by the the increased public awarness towards AIDS. Although there is a decrease in the case doubling time, and the disease is not arrested, the number of individuals affected is still increasing (TABLE II).

TABLE II
Progression of AIDS in selected countries since the onset of the disease

Country	<79	1979	1980	1981	1982	1983	1984	1985	1986
Fed. Rep. Germany	1	2	-	-	10	42	-	377	875
France	6	7	12	17	47	94	-	573	1040
United Kingdom	-	-	-	2	7	24	77	287	610
Switzerland	-	-	2	5	10	17	-	100	192
Belgium	-	-	2	6	14	38	-	139	180
Italy	-	-	-	-	-	-	-	-	367
USA	-	-	-	129	514	4069	9553	16400	29536
Brazil	-	-	-	-	-	50	188	540	754
Canada	-	-	-	-	-	81	217	513	786
Haiti	-	-	-	-	-	232	340	377	501
Zaire	-	-	-	-	-	-	322	832	-

Source:

Bulletin of WHO, 1984; Weekly Epidemiological, Record, 1986; WHO, 1987.

This growth in the incidence of AIDS led the Public Health Service (PHS) of the United States to predict that by 1991 74,000 more Americans will acquire the disease (Anon, 1986). According to this prediction, the total AIDS cases, 4 years from now, in the USA alone will reach 311,000 of whom 179,000 (53%) will die. Outside of Europe and North America detailed information is not available since inadequate health care management and poor diagnostic capabilities make the accurate assessment of AIDS difficult.

Initially, the first AIDS cases were male homosexuals however more detailed investigations showed that other social groups such as intravenous drug users, recipients of blood and blood products, hemophiliaics, children borne to drug addicted mothers and heterosexuals were afflicted with the disease (Curran et al., 1985). In the USA and Europe 73-75% of AIDS patients are homosexuals whereas in Africa, heterosexual transmission of the disease predominates (Curran, et al., 1985; Quinn, et al., 1986; Acheson, et al., 1986).

As can be seen from Table III, in Africa the epidemiological picture is different from that of America or Europe. In Africa, women are frequently affected and the male to female ratio of AIDS patients is nearly equal (Piot and Mann, 1986) whereas in Europe and the USA the ratios are 16:1 and 14:1 respectively. Thus, heterosexual transmission of AIDS most probably indicates that an ever increasing number of individuals may become infected.

TABLE III

PROGRESSION OF AIDS IN SELECTED COUNTRIES SINCE THE ONSET OF THE DISEASE

Risk Groups	USA	Europe	Africa
Cases of AIDS	12932	2162	177
Male:Female	14:1	16:1	1:1.1
Homosexuals	75%	73%	2.5%
IV Drug users	17%	11%	0%
Blood Transfusion	1.5%	2%	4%
Haemophiliacs	0.7%	4%	0%
Heterosexuals	1%	6%	80%
Others	4.8%	4.0%	13.5%

Source: Quinn, et al., 1986.

AIDS has been reported from 27 African countries predominantly among high risk gorups such as prostitutes and their clients (Mann, et al., 1986; WHO, 1986). The number of AIDS cases in some African countries is shown in TABLE IV.

TABLE-IV

AIDS CASES IN AFRICAN COUNTRIES AS OF FEBRUARY 6,1987

Country	No. of AIDS cases
Besatho	1
Benin	2
Botswana	6
Cameroon	21
Central Africa Republic	250
Chad	1
Congo	250
Ghana	73
Ivory Coast	118
Kenya	109
Malawi	13
Mozambique	1
Nigeria	2
Rwanda	13
South Africa	41
Tanzania	699
Tunisia	2
Uganda	766
Zaire*	832
Zambia	250
Zembabwe	6

Source: WHO, 1987; * Quinn, et al., 1986 (1985 data).

Unlike many other diseases which affect the old and the very young, AIDS affects the most productive sector of the population and is therefore a serious social problem (Fig. 2). For example, in Nairobi, Kenya,

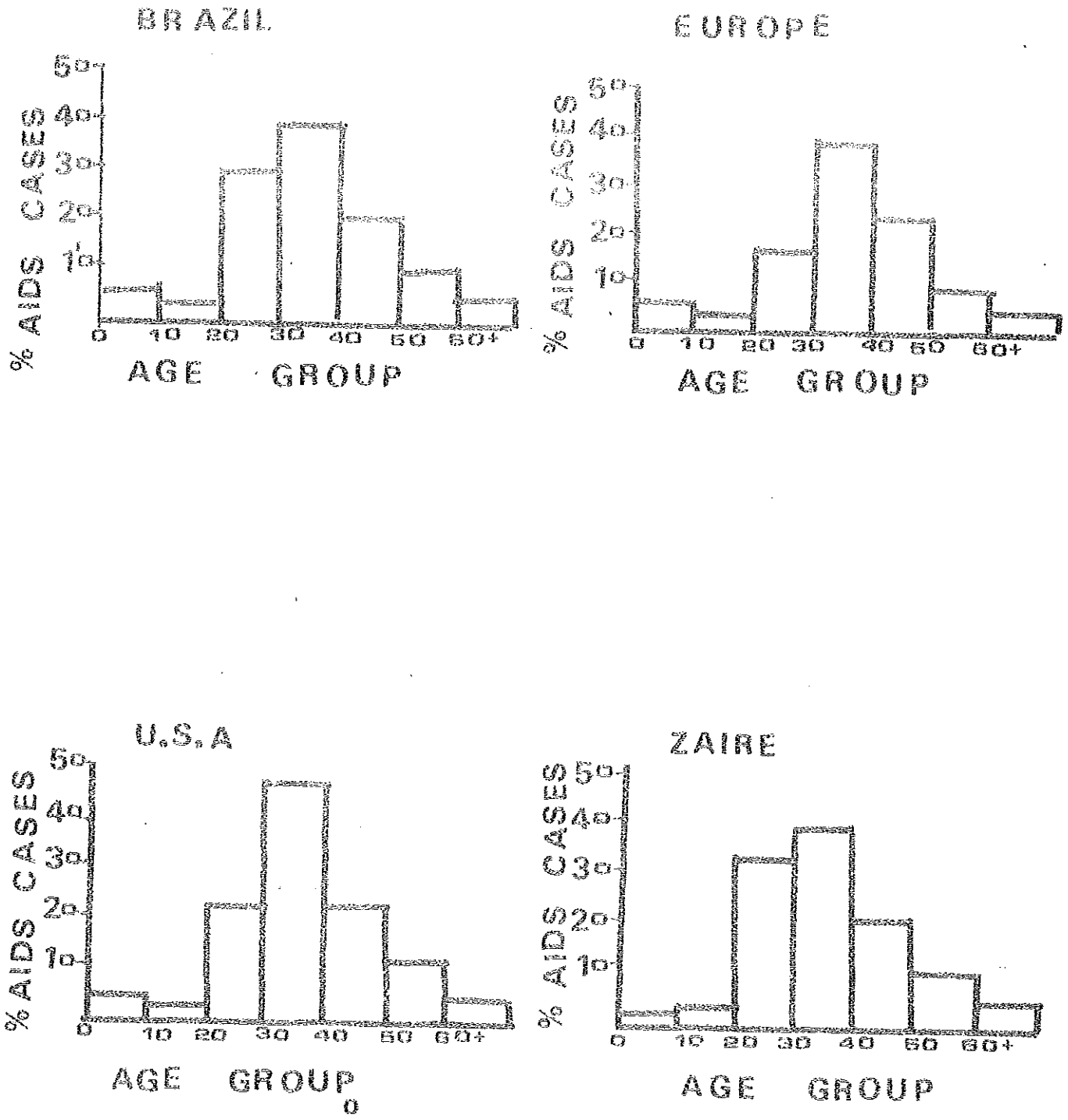


Fig.2. Age Distribution of Confirmed AIDS Cases in the U.S.A., Zaire, Brazil and Europe (Population Report, 1986, Quinn, et al. 1986).

the mean age of the infected individuals is 28 years and in the USA and Zaire 80-91% of AIDS cases are found in people between 20 and 49 years (Kreiss, et. al, 1986; Mann, et al., 1986; and Selwyn, et al., 1986, and this age group is sexually active.

SEROLOGICAL ANALYSIS OF HIV INFECTION

In many viral infections the host produces specific neutralising antibodies which result in protective immunity. However, the antibodies produced in response to HIV infection are non-neutralizing and are only markers of HIV infection (WHO, 1985; Selwyn, 1986). As can be seen from Table V, earlier findings, in 1984, showed that exposure to HIV results in the production of antibodies and both the virus (Schupback, et al., 1984; Sarngadharan, et al., 1984; Gallo, et al., 1984; Brun-Vesinet et al., 1984) (See also TABLE V).

TABLE V

ANTIBODIES TO HIV IN PATIENTS WITH AIDS AND ARC

Diagnosis	Number Tested	HTLV-III+ Antibody detected Number	%
AIDS	49	43	87.6
ARC	14	11	78.6
IV DRUG USERS	5	3	60.0
HOMOSEXUALS	17	6	35.2
NORMAL SUBJECTS	164	1	0.6

Source: Schupbach, et. al., 1984; Sarngadharan, et al., 1984; Gallo, et. al, 1984.

These findings were the corner-stone for the development of reagents for diagnostic purposes as well as for detailed molecular and immunological analysis. As a result, the first generation of diagnostic kits and confirmatory tests such as Enzyme Linked Immunosorbent Assay (ELISA)and Western bloting were developed. As a result of cross reactivity with HLA antigens present on the cell line, the ELISA test may sometimes wrongly identiy antibodies to HIV, so-called false positive results. To avoid or minimize these ELISA false positive results a more specific confirmatory test was mandatory and Western blot serves this purpose.

a) Enzyme Linked Immunosorbent Assay (ELISA)

In this test specific antibodies bind to HIV antigens fixed on the solid phase. After consecutive washing, an antibody to human immunoglobulins, conjugated with an enzyme, like peroxidase is added, and forms a complex with the first antibody. The reaction is visualized using a substrate reagent that generates a yellow or orange color, if antibodies against HIV are present. The intensity of the color developed is proportional to the anti-HIV antibody concentration in the sample.

b) Western blot test

Proteins from disrupted HIV are separated electrophoretically on a sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). Once separated, the pattern is transferred across an electric field onto nitrocellulose paper. The nitrocellulose paper is cut into strips and each strip is tested

with test serum. If the serum contains anti-HIV antibodies, they will bind to the antigen. The strips are then treated with an anti-human immunoglobulin conjugated with an enzyme such as peroxidase and subsequently incubated with a color developer such as 4-chloro-1-naphtol. The appearance of black or pink bands on the strips indicates the presence of antibody specific for certain viral proteins having molecular weights of 24,000, 32,000, 41,000, 53,000, 55,000, 64,000 and 120,000 daltons. The test is considered positive if the antibodies are present which react with the 41,000 dalton envelope glycoprotein (gp) in association with some other viral proteins.

Clinical diagnosis and serological assays showed that in the United States of America and Western European countries the majority of adult AIDS cases (80-90%) continue to occur in homosexuals and intravenous drug users and sexual partners of the persons in these groups. Although the main social groups affected by this virus, in these two continent, are homosexuals there is growing evidence to indicate spread of the virus to heterosexuals. For example in Greece, where AIDS has recently been accounted, 6% of the registered female prostitutes have anti-HIV antibodies and about 20%-50% of the prostitutes in West Germany are infected, most of them drug abusers (Acheson, 1986; Haseltine, 1986).

The picture is very different in Africa where homosexuality is uncommon and most of the HIV seropositive individuals are among heterosexually active men and women. An increasing number of people with AIDS and anti-HIV antibodies has been reported from African

countries among groups with risk behaviour such as prostitutes and their clients (Table VI). For example, most male patients in Rwanda gave a common history of multiple contacts with prostitutes and 43% of the patients were themselves prostitutes (Van De Perre et al., 1985).

TABLE VI

Showing the prevalence of seropositive individuals in 6 African countries

Country	Number positives/Number tested (% positive)					
	Prostitutes	Controls	Customers	Controls	STD Clinics* Females	Males
Kenya	50/90(56)	1/42 (<2)	3/40(8)	1/42(<2)	-	-
Rwanda	29/33(88)	4/33(12%)	7/25(28)	2/27(7)	-	-
Zaire	10/377(27)	-	-	-	-	-
Ivory Coast	29/232(12.9)	22/365(3)				
Zambia	-	-	-	-	9/20(45)	32/119(27)
Tunisia	10% (CP)**	-	-	-	-	-

* STD = Sexually Transmitting Disease

** CP = Clandestine Prostitutes.

Source: Van De Perre, et al., 1985; Kreiss, et al., 1986; Melbye, et al., 1986 and Danis, et. al., 1987.

The finding of a significant number of women prostitutes with antibodies to HIV in the African countries (TABLE VI); the establishment of seropositivity or clinical AIDS in individuals with multiple sexual partners, the nearly equal infection ratio of males to females

(TABLE III) suggests that heterosexualactivity and other unknown factors might also be an important factor in the transmission of HIV. HIV infection in these African countries, using prostitutes and their clients as the main transmittors (Table VI), may spread from one community to the other within a short period of time.

OBJECTIVE OF THE STUDY

To date there are no officially reported cases of AIDS in Ethiopia. However, antibodies to HTLV-I, which is one of the suspected organisms of being ancestral to HIV, has been detected from Ethiopian migrants to Israel (the Falashas) (Ben-Ishai, et al., 1985), although a contradictory result was obtained by Katpas, et al., 1986. Hence, the existance of antibodies to HTLV-I in the Falashas; the appearance of seropositive individuals and AIDS patients in the neighbouring countries, the country's economic and diplomatic relations with various nations and the existance of high number of prostitutes in urban areas make it likely that HIV seropositive individuals and/or AIDS patients might be present in Ethiopia. Because of the high number of sexual partners they have, the prostitutes and male clients are exposed to the sexually transmitted diseases. Therefore the main objective of this work is to determine the prevalence of antibody reactivity against HIV in prostitutes and male clients in Addis Ababa related with age and sex. Serum samples collected from these high risk groups and controls were screened by ELISA and all the anti-HIV antibodies positive sera and an equivalent number of negative sera were assayed by Western blot. These tests for antibodies to HIV are used to identify people who are infected and to guage the prevalence of infection in the sample population which is of a paramount public health importance.

MATERIALS AND METHODS

Study Sera

Sera from 494 subjects were collected between November, 1985 and February, 1987. The individuals participating in the study include prostitutes, male clients of the prostitutes, housewives, peasants, and a few samples from other social groups.

1. Female prostitutes:

   a) 153 serum samples were obtained from prostitutes who presented for routine VDRL testing at two health clinics in Addis Ababa, namely the Arada and Kazanchis clinics.

   b) 24 sera were collected from female prostitutes at Chencha (Gamo Goffa province) - about 600 km. South of Addis Ababa. These sera were of interest because they were obtained in a remote rural area.

   c) Follow up study was done on 20 female prostitutes at an interval of 8 to 12 months.

      However, the migration of prostitutes from one bar to the other makes further follow very difficult.

2. Serum samples from 140 male clients of prostitutes were also obtained from Arada and Kazanchis clinics. The individuals in this group came to the clinics complaining of at least one sexually transmitted disease.

3. Control subjects

a) 177 serum samples were collected randomly from individuals coming to clinics and hospitals seeking medical care other than sexually transmitting diseases. The individuals were from both urban and rural areas.

b) 32 stored serum samples from leprosy patients (collected from 1978/1984) were also tested to see if the infection has established in these individuals, who are highly exposed to various types of infection.

From each subject information on address, sex, age, birth place, whether he/she has had a recent blood transfusion or not, whether he/she has had any injection within the last 2 years, marital status, history of sexually transmitted diseases in the last 3 years, health status and duration of prostitution (if a prostitute) were recorded.

A 10 ml blood sample was taken from each patient and centrifuged at 1500 RPM for 15 min. (using IEC Model PR-J centrifuge). The serum was removed and stored at -20 C. These sera were tested for the presence of anti-HIV antibodies in two stages. First, all sera were examined by Enzyme Linked Immunosorbent Assay (ELISA) using commercially available kits. These kits were kindly given to Professor Sven Britton by Organon Sweden. This test is useful for the determination of quantitative levels of antibodies to disrupted HIV antigens coated on the wells of the microtiter plates. Secondly, all the ELISA positive samples and an equal number of negative sera were further analysed by Western bloting using HIV antigens supplied by Dr. Robert C. Gallo, from the National Cancer Institute, USA to Prof. Sven Britton.

1. Enzyme Linked Immunosorbent Assay (ELISA)

96 well plates coated with HIV lysates with the required reagents were obtained from Organon Teknika. To each well 50 ul of each serum sample, at a dilution of 1:100 in sample diluent (4 ml of sample diluent, 20 ml of normal goat serum and 80 ml of distilled water for 96 wells) was pippetted. The plate was covered with plate sealer and incubated in a wet chamber at 37 C for 30 min. The wells were washed 4 times with wash buffer (concentrated phosphate buffer diluted in 1:25 with distilled water). 40 ul of the conjugate dilution (5.5 ml of diluted sample diluent added to a vial containing freeze dried conjugate) was poured into each well, covered with plate sealer and incubated at 37 C for 30 min. The wells were then washed 4 times with wash buffer and allowed to react with 50 ul of the substrate mixture containing orthophenylene diamine (OPD) (3 OPD tablets in 7.5 ml distilled water) with 300 ul of urea peroxide solution (one urea peroxide tablet in 10 ml of distilled water) and incubated in a dark place at room temperature for 30 min. (this time without plate sealer). The reaction was stopped by the addition of 50 ul of 2M H_2SO_4 in each well and the resulting color measured with a Dynatech ELISA reader (at 492nm).

2. Western Blot

HIV antigens were mixed with the final sample buffer (consisting of 20% glycerol, 10% mercaptoethanol, 4% SDS and 50mM Tris buffer in the presence of bromophenol blue, pH6.8) boiled for 5 minutes, centrifuge at 15,000 RPM for 5 minutes and the supernatant was loaded on the gel. Then the HIV viral antigens were electrophoresed on an 11.5% running

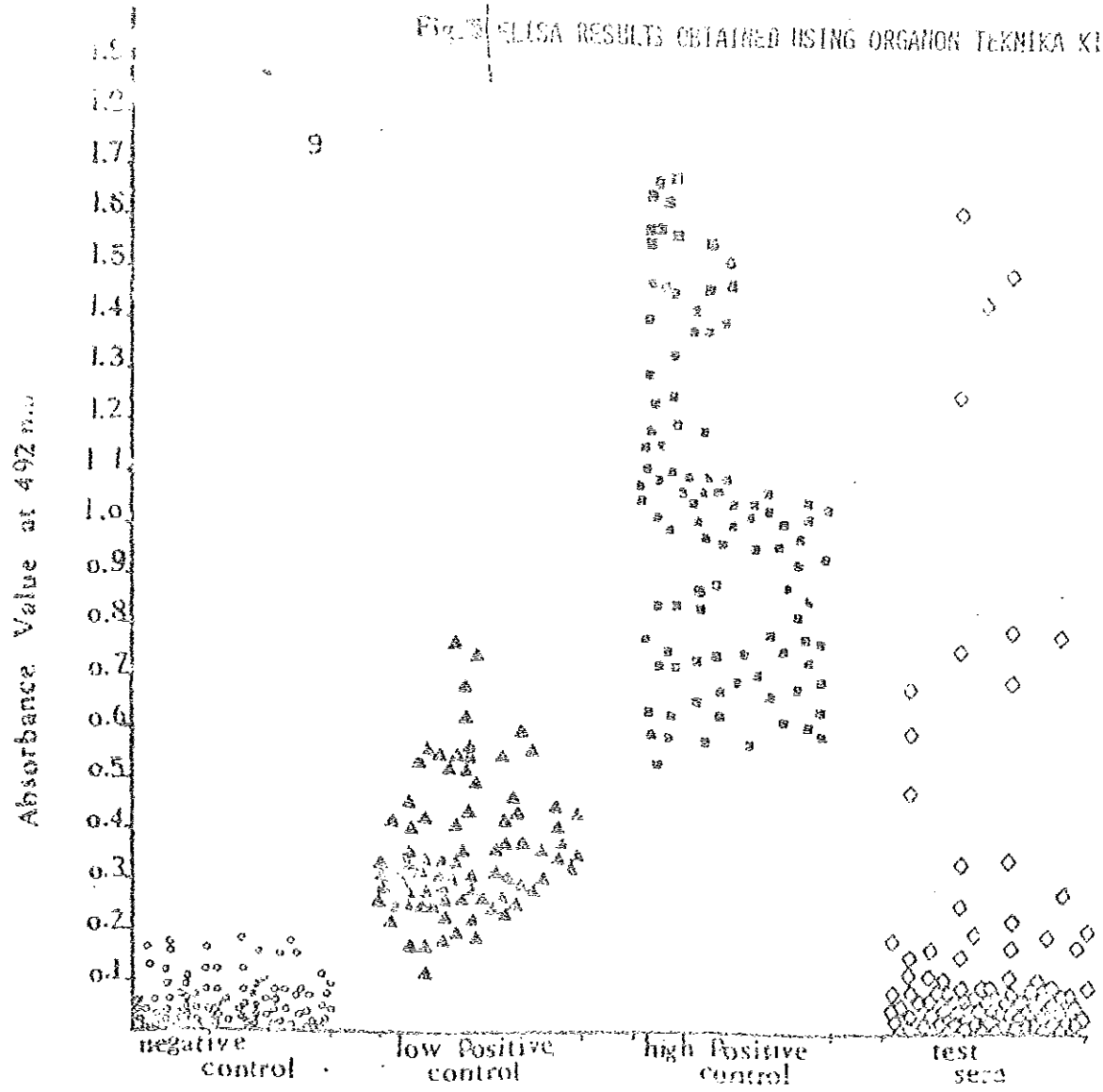
gel and 4% stacking gel, the thickness of the gel being 0.75mm. The gel leading dye front was electrophoresed into the separating gel 13 cm at 15mA. The protein bands on the gel were then transferred to a nitrocellulose paper in a trans-blot aparatus (BIORAD) in Western blotting buffer (30mM Tris and 250mM glycine, ph8.3) at 42 volts for 45 minutes. The nitrocellulose sheet was cut into 0.5 - 0.6 cm strips and blocked with 0.05% Brij (Polyoxyethylene 20 cetylether (Brij 58)-SIGMA) in 50 mM tris base containing 0.15 M NaCl (pH7.4)-MERCK - for 60min. The test sera were then diluted in 1:100 with the blocking medium. The strips were incubated with the diluted sera overnight with rocking. All the incubations were performed at room temperature. The strips were washed 3 times with 0.05% Brij solution and treated in a 1:150 dilution of peroxidase conjugated rabbit anti-human immunoglobulins and incubated for 2 hours (with rocking). This was followed by 3 times washing. To amplify the bands, the strips were further incubated with peroxidase labelled swine anti-rabbit immunoglobulins (1:150 dilution) and incubated for 60 minutes. After washing the strips 4x in 0.05% Brij followed by rinsing in distilled deionized water for 5-10 min., the bound peroxidase was detected with 4 - chloronapthol 50mg of 4- chloro-napthol disolved in 10 ml of dimethyl sulphoxide . From this solution 2 ml was taken and added 100 ml. of deionized water. To this solution 2 ml of a 1:100 dilution of a 30% hydrogen peroxide (V/V) was added and the strips were flooded with this solution. The strips were shaken vigorously and within 2 to 4 minutes the characteristic banding pattern showed up on the strips incubated with a positive serum.

RESULTS

Sera from 494 subjects (mean age 27.9, range 12-75 years) including prostitutes, male clients to prostitutes and control groups were collected, and screened by ELISA (Organon Teknika) for the prevalence of anti-HIV antibodies. An ELISA positive or doubtful serum was repeated two times before it is analysed by Western bloting. If the serum is positive twice then it is considered as ELISA positive and tested by the confirmatory test - Western blot. Although, the manufacturer of the kit recommends the cut off value as an absorbance reading of greater or equal to 0.5 times that of the mean absorbance value of the negative controls plus the mean absorbance value of the low positive controls (usually gives a value ranging from 0.11-0.35), in practice almost all the positive sampels show a value ranging from 0.33 to 1.87 (see Fig. 3). All the anti-HIV antibody positive and few selected seronegatives sera were further assayed by Western blotting. An individual was considered seropositive for HIV if the Western blot assay showed a positive result for the gp41.

Prostitutes comprised 177 of the 494 individuals included in the study (TABLE-VII). One hundred and fifty three prostitutes (mean age 25.2, range 15-58 years) live in Addis Ababa (urban) and the remaining 24 (mean age 23.08, range 17-40 years) are inhabitants of Chencha, Gamo Gofa province, (Rural). The overall mean age for both groups was 24.96 years and duration of prostitution ranges from 2 months to 12 years. Only one of the prostitutes had sexual contact with non-Ethiopian citizens and two had had taken blood transfusion in the last four years.

Fig. 9 ELISA RESULTS OBTAINED USING ORGANON TEKNIKA KITS.



One hundred and forty men who were attenders of two clinics which treat sexually transmitting diseases were also enrolled in the study. These individuals are among the 494 study subjects and treated as clients of prostitutes. Hundred thirty of these men (mean age 26.9, range 15-68 years) came from urban areas for medical treatment of venereal diseases. The overall mean age of the clients was 27.13 years. All the clients had had more than one sexual partner and no one had a history of blood transfusion or sexual contact with non-Ethiopians.

TABLE VII

Prevalence of Anti-HIV antibodies in Prostitutes, male clients and control groups

		STUDY GROUP							
		Prostitutes		Clients		Controls			
		Urban	Rural	Urban	Rural	Urban		Rural	
						Male	Female	Male	Female
No. Sera tested		153	24	130	10	50	72	34	21
Age (yrs)	Mean	25.25	23.08	27.13	30.7	28.02	29.6	34.1	35.76
	Range	15-58	17-40	15-68	22-75	15-50	15-68	17-75	18-65
Number of Anti-HIV Antibody Positive Sera	ELISA (%)	9(5.9)	0	6(4.6)	1(10)	0	2(2.8)	0	0
	Western Blot (%)	9(5.9)	0	4(3.08)	0	0	0	0	0

As shown TABLE VII 177 individuals were enrolled in the study as controls. Among them 112 (mean age 29, range 17-75 years) are from

Addis Ababa (urban) and 55 (mean age 34.7, range 17-75) are from rural areas.

Serological Tests - TABLE VII and TABLE VIII shows the prevalence of anti-HIV antibodies in all subjects as tested under different assays. From Table VII and VIII it is shown that anti-HIV antibody frequency was higher in prostitutes than clients or control subjects. Based on the Western blot assays antibodies against HIV were detected in 9 (5.8%) of the prostitutes and 4 (3%) of the male clients who are living in Addis Ababa. (See also fig. 4). None of the samples tested from the rural areas were immunoblot positive.

TABLE VIII

Anti-HIV antibody positive sera tested with different assays

	Organon Test	Welcome* Test	HIV antigen detected in kD (Western blot)	
001	+	+	24,34,41,53,55,64	+
0071	-	+	24,34,55	-
0100	+	+	24,55	-
0104	+	+	24,34,41,53,55,64	+
0119	+	+	24,34,41,53,55,64	+
0122	+	+	24,34,41,53,55,64	+
0167	+	-	24,55,64,	-
0254	+	+	24,34,41,53,55,64	+
0287	+	+	24,55	-
0317	+	-	negative	-
0406	+	?	24,34,41,53,55,64	+
0515	+	?	24,34,41,53,55,64	+
0545	+	?	24,34,41,53,55,64	+
0548	+	?	24,34,41,53,55,64	+
0552	+	?	24,34,41,53,55,64	+
0553	+	?	24,34,41,53,55,64	+
0554	+	?	24,34,41,53 55,64	+
0212	+	+	24,34,41,53,55,64	+

* Test performed in the State Bacteriological Laboratory, Sweden.
? Serum not tested.

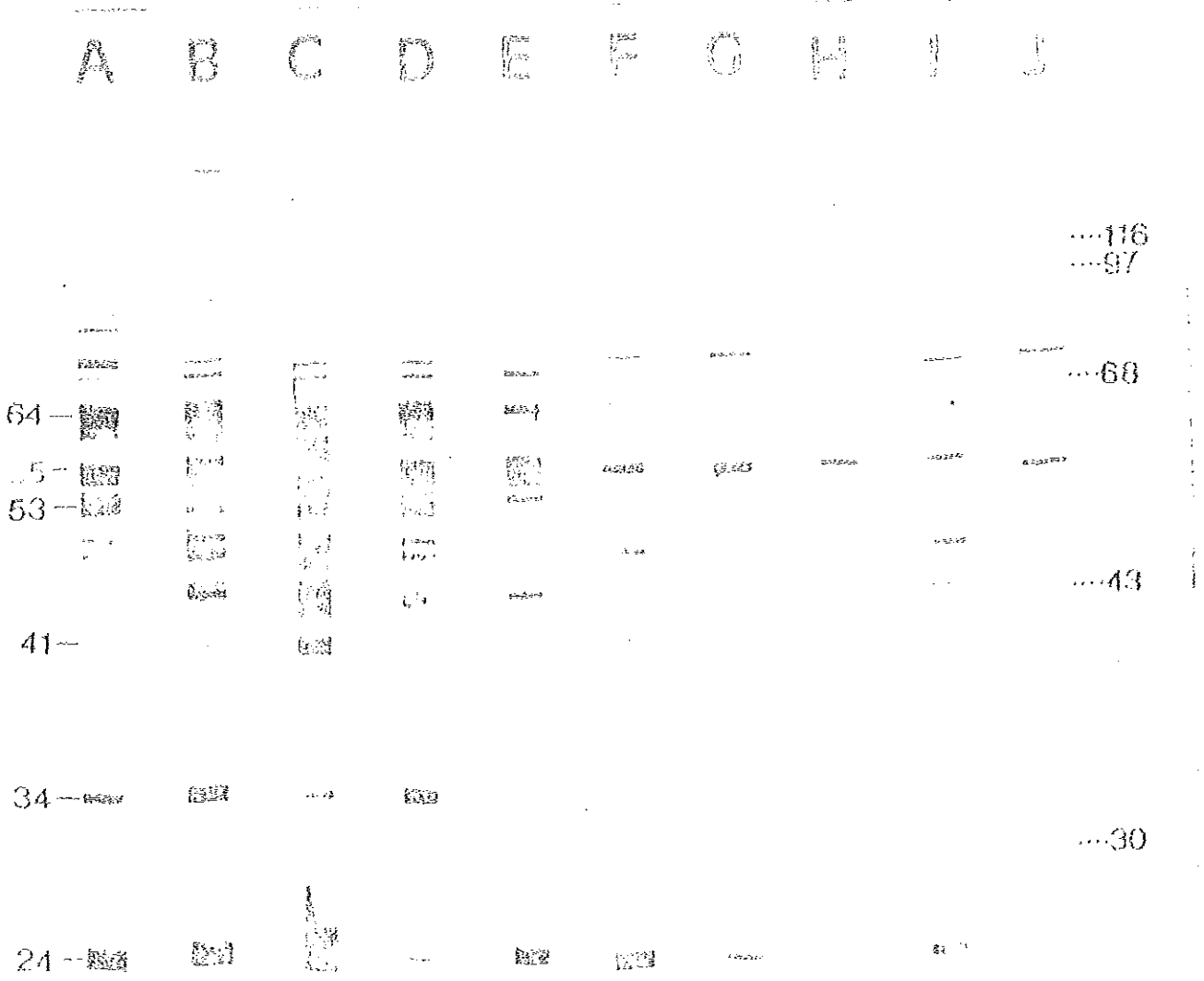


FIG.4

ANTIBODY REACTIVITIES OF DIFFERENT SERA TESTED BY WESTERN BLOT. LANE A-C SHOWS BOTH ELISA AND WESTERN BLOT POSITIVE SERA, LANE F-H ELISA POSITIVE WESTERN BLOT NEGATIVE SERA; LANE I, ELISA NEGATIVE BUT POSITIVE FOR p^{24} AND p^{53} ON WESTERN BLOT; LANE J, ELISA AND WESTERN BLOT NEGATIVE SERA.

Age distribution of the seropositive subjects - The age distribution of urban individuals with anti-HIV antibodies range between 15-49 years (TABLE IX). 91.1% of the seropositive subjects have an age range of 20-49 years.

TABLE IX

Age distribution of anti-HIV antibody positive subjects in the Urban study group

Age Group	Number of HIV Seropositive Sera in Western Blot (%) Prostitutes	Clients	Controls
15-24	5 (3.3)	-	-
25-34	2 (1.30)	3(2.31)	-
35-44	1 (1.65)	-	-
45-54	1 (0.65)	1 (0.76)	-

Regarding the clinical history and status of the 7 available seropositive individuals only one male clients showed clinical features of AIDS or ARC. Others show no marked difference in their clinical history and status when compaed to the seronegative individuals. The results are presented in TABLES X and TABLE XI.

TABLE X

HISTORICAL AND CLINICAL STATUS OF 4 ANTI-HIV ANTIBODY POSITIVE PROSTITUTES

Code No./ *Age	Clinical History and Status
0212/ *17	Prostitute for 1 year, at least 10 sexual contacts per month (10-15 birr for a night and 6 birr for a time) has contacted gonorrheae and urinary tract infection during the period of prostitution. Multiple erosion of the vagina and ulcer with scanty discharge. Her weight decreased from 65-57 kg. within a year. She also complains of fever. No sexual contact with non-Ethiopians.
0122/*22	Prostitute for 3 years, sexual contact with 8-10 customers per month (10-20 birr for a night and 5 to 10 birr for a time). No sexual contact with non-Ethiopians. Healthy individual with no complaint of any disease.
0119/*24	Prostitute for 7 years, sexual contact with 14 customers per month and usually practices sexual intercourse 2 times per day (10-20 birr per night and 5-10 birr for a time). She had contacted chancroid, and no sexual contact with a non-Ethiopian. Complaining of constant fever.
0254/*24	Prostitute for 8 years, frequence of sexual contact 1-3 times per day, before two years her frequency of sexual contact ranged between 30-90 per month. But later it was slowed down to 3 sexual contacts per week. Has vaginal discharge and complaining of intermitant fever and infrequently night sweeting. No sexual contact with non-Ethiopians. Weight loss from 64 kg. to 59 kg. with in 8 months.

TABLE XI

HISTORICAL AND CLINICAL STATUS OF 3 ANTI-HIV ANTIBODY POSITIVE MALE CLIENTS OF PROSTITUTES

Code No./ *Age	Clinical History and Status
0553/*45	Married, father of 4 children, no history of blood transfusion, has a marked weight loss from 62 kg. to 51 kg. within 6 months. Developed Herps zoster infection for over 6 months.
0554/*30	Single, without children, no history of blood transfusion, showed marked weight loss from 55-36 kg. within 6 months time. He has been treated for chancroid before one year. Has sexual contact with about 10 prostitutes in the last 2 years.
0552/*27	Single, nwithout children, no history of blood transfusion. Frequently visits prostitutes. Has a non-Ethiopian girlfriend. Except his complaint of an abdominal pain, he is healthy looking.

We attempted to investigate the risk of transmission of HIV to non-sexual household contacts of 5 seropositive individuals. Twenty seven participants have had casual contact with these seropositive subjects. The type of casual contact includes hand shaking, hugging, kissing, sharing of toilets, bath, towels, rathor blades, eating and drinking utensils and sleeping in the same bed. The mean age of the participants was 23.6 ranging from 2-58 years and non had antibodies to HIV virus.

To examine the establishment of HIV infection in Ethiopia before 1984 and to see if there is any correlation between leprosy and HIV infection we did a retrospective study on 32 stored sera collected

between 1978 and 1984. The first ELISA test showed one sample with weakly positive result but when the sample was retested it was found to be negative which was supported by Western blot assay.

DISCUSSION

In Africa the at risk behaviour groups most susceptible for HIV are mainly male clients who frequently visits prostitutes and the prostitutes themselves (Clumeck, 1985). A high prevalence of antibodies to HIV has been reported from Rwanda, Kenya and Zaire (Vander Perre, et al., 1985; Kriess, et al., 1986; and Mann et al., 1986). Clinical AIDS is also recognized in African patients, heterosexual contact being the most probable mode of transmission (Brun-Vezinet, 1985; Piot and Mann, 1986). Serological surveys for anti-HIV antibodies done in Africa were initially unreliable because of high false positive rates (Mann, et al., 1987). In our study a positive or doubtful sera for HIV antibody was first screened by ELISA two times and then for a repeatedly ELISA positive sera a Western blot assay was performed. Even by repeated screening of the serum sample by ELISA alone we were able to exclude 5 sera which showed a positive result in the initial screening. Calculations based on the company's (Organon Teknika) recommendation mostly showed an absorbance reading with a range of 0.11 - 0.35. However, in practice all the Western blot positive samples showed a value ranging between 0.33 to 1.87 (see Fig. 3).

The result of the Addis Ababa sera showed (TABLE VII) that the prevalence of antibodies against HIV infection was 5.8% in prostitutes and 3% in clients. Even if we consider the ELISA positive results alone, the prevalence of HIV antibodies were more common in prostitutes than the client or controls subjects. Nevertheless, the percentage of seropositive prostitutes in Addis Ababa is low when compared with the results from Kigale, Rwanda (88%), Nairobi, Kenya

(59%), and Kinshasa, Zaire (27%). The reason for this differences is not clear although the low number of seropositive individuals within the urban population tested and the lack of sero-positive individuals from rural areas (TABLE VII) may suggest a more recent establishment of HIV infection within the two high risk groups studied in Addis Ababa.

There is also evidence that stored sera collected from Ethiopia between 1978 and 1984 contain no anti-HIV antibody (Data not shown). Maayan et al., 1986 has also tested 244 recent immigrants from Ethiopia to Israel using Abott ELISA for the presence of HIV antibodies. By this test about 16.4% were seropositive and when the confirmatory test was done using Western blot all were found to be seronegative. The League of Red Cross and Red Crescent Societies (1986) and the WHO experts in Geneva (Engers - personal communication) have also shown the lack of anti-HIV antibodies from Ethiopian sera. These researchers did not mention which social groups the sera belonged and hence a direct comparison may not be possible with our results. All these may suggest that HIV infection has not yet spread in Ethiopia.

The relatively high prevalence of HIV infection in urban prostitutes when compared with clients and controls may also be an indicator of the HIV's potential spread within a few years time to another urban areas of the country facilitated by the movement of prostitutes from one area to the other. Additionally, this prevalence of anti-HIV antibodies in prostitutes and clients rather than the control groups may suggest a clue for the heterosexual transmission of HIV among the promiscuous individuals.

All ELISA positive samples and nearly an equal number of ELISA negative samples were tested by Western blot assay. Sera showing borderline or doubtful values in ELISA were further confirmed by Western blot. The predominant HIV proteins detected (Table VIII) include polypeptides having molecular weights of 24,000, 34,000, 41,000, 53,000, 55,000 and 64,000. Sarngadharan, et al., (1984) have shown a glycoprotein (gp) with a molecular weight of 41,000 daltons (gp41) to be the prominent and commonly recognized protein by sera from AIDS patients and he suggested that a sample is immunoblot positive if it showed a banding pattern in the 41,000 molecular weight region in association with one of the major HIV proteins. But researchers still differ in opinion as to which HIV protein has to be considered as a marker for HIV infection. The observation by Lange et al., 1986 showed the sequential production of antibody in the course of HIV infection. The protein with 24,000 daltons molecular weight (p24) was detected first followed by p55 and finally appearance of antibodies to the rest of the HIV proteins, complicates the problem.

According to Groopman et al.,(1986) a specimen is said to be immunoblot reactive by the presence of bands at the p24 or gp41 molecular weight region in the presence or absence of other associated bands. Whereas Kreiss et al., 1986 considered a serum sample to be positive if a p24 positive band appears on the blot. Because of the similarities in the core protein of HIV-I, HIV-II and other human retroviruses (Gallo, 1985; Wong-Staal and Gallo, 1985), I prefer to consider a sample to be seropositive if the sample tested shows a prominent reactivity with the virus's trans-membrane glycoprotein, gp41, in association with other HIV viral proteins.

Based on this criteria out of the 18 ELISA positive sera 13 were confirmed to be positive by Western blot (Table VIII). From the Western blot negative but ELISA positive sera 4 were positive for p24 and p55 (See Fig. 4). These ELISA positive and immunoblot negative sera may be due to the presence of cross reacting antibodies as a result of infection by related viruses. The observation of Lange et al., (1986) which emphasizes the appearance of antibody to p24 as an initial response in the course of HIV infection may not be ruled out. The 3rd possibility could be a non-specific binding.

Seropositivity was restricted to individuals who were in their sexually active period of life. About 91% of the seropositives are within the range of 20-49 years. (Table IX) and 77% of the seropositives are in the range of 20 to 40 years. Similar results have been reported from Zaire and USA (Quinn et al. 1986). The peak prevalence occured earlier in women (range between 15 and 24 years) than in men (range 25-34 years) and the infection ratio between female and male being 2.5:1.

Our study was mainly concentrated on screening of individuals coming to clinics treating sexually transmitted diseases and hence it may be difficult to see AIDS or ARC cases. As can be seen from TABLE X and Table XI there was no sign of lymphadenopathy which is usually observed in more than 50% of AIDS or ARC patients. Out of the seven anti-HIV antibody positive individuals examined for clinical history and status (Table X and XI), 3 complained of fever, 4 showed weight loss which was marked in two. One seropositive male client, in addition to a marked weight loss, had recurrent herps zoster, which is one of the opportunistic infections shown in AIDS or ARC patients, and

hence can be considered as ARC patient. Except this individual, whether the complaints of the seropositive individuals are results of HIV infection or not requires further follow up.

Once we established the prevalence of anti-HIV antibodies in the prostitutes and their clients, we tried to see the risk of transmission of HIV among friends, sisters, brothers and other relatives of 5 seropositive subjects. Our result showed that all the samples collected from these subjects were negative. The limitations of this study is that we have no information on how long the seropositive individuals have been carriers of HIV before diagnosis. But in isotype assay (data not shown) all the seropositives show high banding pattern with IgG antibody rather than IgM antibody, which may indicate that infection is not recent in these subjects. These results agree with the earlier findings reported by Saltzman, et al., (1986) and Peterman et al., (1986) and from our result we have no evidence of transmission of HIV through casual contact.

Although the prevalence of anti-HIV antibodies does not appear to be wide spread throughout the population, our findings clearly indicate the establishment of HIV infection in prostitutes and male clients. A better understanding of the distribution and route of spread may be an important factor in the prevention of HIV infection.

To slow down the progression of HIV, it is worth mentioning the following points.

a) epidemiological surveillance to find out risk factors and risk groups.

b) Screening of blood donors among the blood donors in order to keep contaminated blood out of the blood supply.

c) Follow up for all the seropositive individuals.

d) Educate people about AIDS and encourage them to limit the number of sexual partners.

e) Avoid sexual intercourse with an individual who has multiple sexual partners.

f) Educating people and increasing of their awareness towards AIDS.

g) Establishment of well equipped laboratory which will have a capacity for serological diagnosis of HIV infection.

Momentarly in the absence of vaccine or therapeutic drug, for HIV infection, there is only one way to go out of this pandemic - self prevention.

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