IMMUNOLOGICAL MARKERS OF DISEASE PROGRESSION IN HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 INFECTION IN DAR ES SALAAM, TANZANIA.

WILLY SHILEANGA KIKOKA URASSA

MD (Dar), MSc (Karolinska)

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE (MICROBIOLOGY) OF THE UNIVERSITY OF DAR ES SALAAM.

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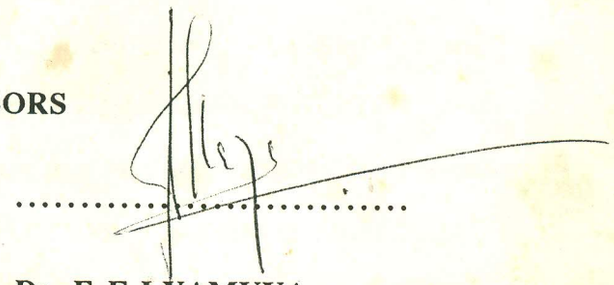
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Summary.

A cohort of HIV-1 seronegative individuals who were referents of HIV-1 seropositive individuals has been followed up for four years to study the sex difference and variability of lymphocyte subsets and levels of B-2 M. Lymphocyte subsets determination was done using flow cytometry and levels of B-2M were determined using an ELISA. Females had significantly higher mean $CD4^+$ T-lymphocyte counts and CD4:CD8 lymphocyte ratios but significantly lower $CD8^+$ T-lymphocyte counts and concentration of B-2M concentration than males. Percentage $CD4^+$ and $CD8^+$ T-lymphocytes were more reproducable than absolute counts. There was lack of correlation between percentage and absolute $CD4^+$ T-lymphocyte counts. Among the individuals $CD3^+$ T-lymphocytes percentage, $CD4^+$ T-lymphocyte counts, $CD8^+$ T-lymphocyte counts and percentages and concentrations of B-2M varied significantly in males in the different years of followup . However, in females only percent total lymphocytes and $CD3^+$ T-lymphocytes varied significantly. Total lymphocyte counts, percent $CD4^+$, $CD8^+$ T-lymphocytes and CD4:CD8 lymphocyte ratios did not vary significantly in both sexes during the follow-up period. It is therefore important to consider the variations of these prognostic markers in normal individuals in relation to time and sex when evaluating these parameters in HIV-1 infected individuals.

Haemoglobin levels, total peripheral white blood cell count, total lymphocytes, T-lymphocyte subsets and B-2M concentrations were determined in 183 HIV-1 infected Tanzanian individuals in different clinical stages of HIV infection and in 119 healthy HIV-1 seronegative Tanzanian blood donors. Haemoglobin concentration, $CD4^+$ T-lymphocyte percentages and counts, $CD8^+$ T-lymphocyte percentages, CD4:CD8 lymphocyte ratios and concentration of B-2M were strongly correlated with clinical stages of HIV-1 infection. However, total peripheral WBC counts, total lymphocyte counts, total T-lymphocyte counts and $CD8^+$ T-lymphocyte counts were not

significantly different among individuals in the various clinical stages of HIV-1 infection. In a longitudinal study, hotel workers were followed up for a period of four years to study the natural history of HIV infection. Among individuals with known approximate dates of HIV-1 seroconversion, there was a rapid fall of $CD4^+$ T-lymphocytes, CD4:CD8 ratios and a rapid rise of the $CD8^+$ T-lymphocyte counts in the first year after HIV-1 seroconversion. There was also a gradual rise of B-2M levels. The concentration of soluble CD30 doubled during the first year after seroconversion and remained high during the follow up period. Therefore, $CD4^+$ T-lymphocyte counts and concentrations of soluble CD30 and B-2M showed uniform changes during the follow up period of HIV infected individuals indicating that these parameters are important early markers of disease progression.

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- Swedish Agency for Research Cooperation with Developing countries for supporting the HIV infection programme in Tanzania within which the work for this Dissertation was undertaken.

DECLARATION

I Willy Shileanga Kikoka Urassa, declare that this dissertation is the product of my work and no part of this dissertation has appeared in any other publication for a degree in any other University.

Signed..... W Urassa .....
Candidate
Date ..... 24/10/1995 .....

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DEDICATION

This dissertation is dedicated to my dear father and mother Shileanga and Arafumandumi for ensuring that I grew up strong enough to face the world.

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ABBREVIATIONS:

ADCC	antibody-dependent cell-mediated cytotoxicity
ADE	antibody directed enhancement
AIDS	Acquired immunodeficiency syndrome
B-2M	beta-2 microglobulin
CD	cluster of differentiation
CDC	Centres for Disease Control
cDNA	complementary deoxyribo nucleic acid
CTL	cytotoxic T lymphocyte
EBV	Epstein-Barr virus
EDTA	Ethanolamine diamine tetra acetic acid
ELISA	enzyme linked immunosorbent assay
Fc	crystallisable fraction
gag	group antigen
FITC	fluorescein isothiocynate
gp	glycoprotein
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HTLV	human T-lymphotropic virus
IN	intergrase
LAV	lymphadenopathy-associated virus
LTR	long terminal repeat
kb	kilobyte
IFN	interferon
IL	interleukin
MHC	major histocompatibility complex

mRNA	messenger ribonucleic acid
nef	negative regulatory factor
NK	natural killer
sCD30	soluble CD30
SLE	systemic lupus erythematosus
SIV	simian immunodeficiency virus
T	thymus derived lymphocytes
Tat	trans-activating factor
Th	Thymus derived helper cell
OHL	oral hairy leucoplakia
p	protein
PCR	polymerase chain reaction
PE	Phycoerythrin
pol	polymerase
pr	protease
ORF	open reading frame
RT	reverse transcriptase
WHO	World Health Organisation
vif	viral infectivity factor
vpx	viral protein x
vpu	viral protein u

1.0. Introduction.

Acquired immunodeficiency syndrome (AIDS) is characterized by life threatening opportunistic bacterial, viral, parasitic and fungal infections and neoplasms that culminate into a progressive loss of the body's ability to mount effective immune response. It is now well established that this syndrome is caused by two related primate lentiviruses; human immunodeficiency virus types 1 (HIV-1) and 2 (HIV-2) as reviewed extensively by Levy and Fauci[1,2]. This syndrome became apparent in the middle of 1981 when an unexpected occurrence of *Pneumocystis carinii* pneumonia and Kaposi sarcoma among young gay men were reported in California and New York[3]. Subsequent reports in 1981 and 1982 identified persons with haemophilia, recipients of blood and blood products, intravenous drug users and their heterosexual partners and children who had similar opportunistic conditions associated with unexplained immunodefiency[4,5]. After some surveillance studies, beginning in 1982 this immune disorder and accompanying illness became known as the acquired immunodeficiency syndrome (AIDS). HIV-1 was first isolated in 1983 in France at the Pasteur Institute by Barre-Sinoussi et al[6] and this was confirmed in the United States by Gallo et al at the National Institute of Health[7]. The French and American groups named the virus as lymphadenopathy associated virus (LAV) and human T-lymphotropic virus type 3 (HTLV-III) respectively. In 1986 a related virus, human immunodeficiency virus type-2 was isolated and identified as the cause of AIDS in West Africa[8,9].

Since the first description of AIDS in 1981, the number of people with HIV infection and with AIDS has steadily increased globally and has recently spread to South East Asia[10]. Worldwide, over 1,169,811 cumulative AIDS cases in adults and children had been reported to the World Health Organization (WHO) by 30th June 1995, but an

estimation of over 4.5 million AIDS cases had occurred[10]. It was also estimated that as of mid-1995 around 18.5 million adults and more than 1.5 million children had been infected with HIV since the beginning of the pandemic[10]. In Europe and North America, haemophiliacs, intravenous drug users and homosexual men were the initial risk groups of the epidemic, but soon after, the epidemic spread to the heterosexual population who at present account for a majority of infection in the world especially in developing countries. Most of HIV infections in the world are due to HIV-1.

The epidemiology of HIV infection in Africa varies within the continent and is not completely understood. HIV-1 is found in all African countries while HIV-2 is mainly found in West Africa and to a certain extent in Angola and Mozambique[11,12]. Patients with AIDS were first diagnosed in Equatorial Africa in 1981 at roughly the same time as the epidemic was recognised in the USA and Europe[11,12]. HIV-2 infection has been shown to have a more slow progression from the time of infection to AIDS as compared to HIV-1 infection[13]. In Africa high prevalences of HIV infection are commonly found in urban compared to rural areas. Highest infection rates are seen among men and women aged between twenty and forty years, female prostitutes, people with sexually transmitted diseases (STDs) and certain occupational groups such as long distance truck drivers[11,12]. This peculiar pattern of HIV infection is certainly different compared with what has been described in Europe and North America. The rising HIV prevalence in rural areas is partly due to migration of those already infected from the urban areas to rural home areas.

The high prevalence and rapid spread of HIV infection in Africa has been strongly associated with high prevalence of STDs especially genital ulcer disease[14-18]. Nevertheless, some experts argue that other biological factors like being uncircumsized

also play some role in the transmission[18-20]. The magnitude of the epidemic in Africa is therefore high in terms of morbidity and mortality and has social, economic and cultural impacts in the society as a whole. More than 80% of all HIV infections in Africa are acquired through the heterosexual route which is a relatively in-efficient mode of HIV transmission between asymptomatic sexual partners of infected men and women in the absence of other risk factors[21]. Mother to child transmission of HIV-1 accounts for about five percent of infected cases in Africa with transmission rates as high as 30-44% as compared with less than 20% in the USA and Western Europe[22,23]. The parenteral route of transmission accounts for one to five percent of new cases in Africa and is mainly due to transfusion of unscreened HIV-1 infected blood which is mainly given to women of child bearing age and children[14].

In Tanzania cases of AIDS were reported for the first time in Kagera region in 1983[24] where the disease was nicknamed "Juliana" or "slim disease". Since then rising numbers of individuals with HIV-1 infection and AIDS have been reported from all administrative regions of the country. Sero-prevalence and sentinel surveillance studies have shown increasing prevalence of HIV-1 infection in Tanzania especially in at risk populations including pregnant women, long distance truck drivers and bar workers[25-28]. By June 1995, 53,247 AIDS cases had been reported to the Ministry of Health and projections of 2.4 million HIV-1 infections by the year 2000 have been made[29]. In general the epidemiology of HIV-1 infection in Tanzania does not seem to be different from that of the rest of Sub-Saharan Africa except that cases of HIV-2 infections have so far not been detected despite the fact that Mozambique which shares a border with Tanzania has several such cases[29-32]. Therefore the number of HIV infected individuals is increasing rapidly globaly without effective treatment or vaccine against the infection.

There is a need to understand the natural history of HIV infection in our population. Regarding prognostic markers of HIV infection, very few studies can be cited in Sub Saharan Africa which has more than 70% of all HIV-1 infected individuals in the world. In order to use the various prognostic markers sensibly there is a need to determine the reference values of these markers in the population. The present studies therefore aimed at studying the variability of several immunological prognostic markers among HIV infected adults in comparison to non-HIV infected referents.

2.0. Literature review.

2.1. The human immunodeficiency type-1 virus:

The human immunodeficiency virus type 1 is a primate lentivirus belonging to the family *Retroviridae*. Retroviruses are RNA viruses which are unique in that they have an enzyme reverse transcriptase (RT) which transcribes viral RNA to DNA before replication cycle starts, a property which is not seen in other RNA viruses[33].

Lentiviruses are a group of viruses linked together by similarities in genetic composition, molecular mechanisms of replication and in biological interaction with the host [34,35]. Other members of the group are simian immunodeficiency virus (SIV), visna virus (sheep) and many others infecting a wide variety of animals and birds[34]. These viruses cause diseases characterized by long periods of subclinical illness and result later into severe immunodeficiency and multiple organ failure. Lentiviruses persist indefinitely in their hosts and replicate at variable rates during the course of the life long infection[34,35]. Some of the important characteristics of infection with the lentiviruses can be summarized into:

1. Clinical:

-Association with diseases with long incubation periods.

-Association with immune system deterioration.

-Involvement of the haemopoietic system.

-Involvement of the central nervous system.

-Association with arthritis and autoimmunity.

2. Biological:

-Host species-specific and non-oncogenic.

-Cytopathic effect in certain infected cells forming syncytial multinucleated cells.

-Infection of macrophages which act as reservoir.

-Accumulation of unintergrated circular and linear forms of viral cDNA in infected cells.

-Latent or persistent infection in some infected cells.

3. Molecular:

-Large genome(>9kb).

-Truncated gag gene thus having several processed gag proteins.

-Highly glycosylated envelope proteins.

-Polymorphism particularly in the envelope region.

-Novel central open reading frame in the viral genome that separates the polymerase and envelope regions.

-Presence of accessory/regulatory genes.

The characteristics mentioned above have been shown to account for the major pathological and clinical courses of HIV-1 infection and AIDS.

2.2. HIV-1 virion:

The structure of the HIV-1 virion as seen in an electron microscope is characteristic of a lentivirus. It has a cone shaped core composed of the Gag (group antigen) protein which consists of p24, a major structural protein and p17 which is the matrix protein [35-37] (Figure 1). Inside the capsid are two identical RNA strands which are closely associated with the viral RNA-dependent DNA polymerase (Pol) also called the reverse trancriptase (RT) (p66, p53) and the nucleocapsid proteins (p9, p6)[2,35-37]. The viral surface is characteristically made up of 72 knobs containing glycoproteins. The glycoproteins are derived from a 160,000 MW precursor which is cleaved inside the cell by cellular enzymes into a gp120 external surface envelope protein (env) and a gp41 trans-membrane protein[35-37]. The virion gp120 located on the virus surface contains the binding site for the cellular receptor(s) and the major neutralizing

domains[35-37]. The genome of HIV-1 is 9.7 kilobases (kb) in size with open reading frames and is characterized by its high complexity[2]. It comprises two flanking long terminal repeats (LTRs) as well as the three characteristic retroviral structural genes gag, pol and env[35-37] (Figure 1). The LTR have been found to play a primary role in initiating and controlling transcription of the viral proteins in association with other regulatory gene products[2]. There are six additional open reading frames (ORFs) named viral infectivity factor (vif), viral protein r (vpr), transactivating factor (tat), regulatory factor (rev), viral protein u (vpu) and negative regulatory factor (nef) which are present on the viral genome[2,35]. In HIV-2 the gene for vpu is missing and it is replaced by viral protein x (vpx).

The primary transcript of HIV-1 is a full length viral mRNA which is translated into the gag and pol proteins[38]. The gag precursor p55 gives rise by proteolytic cleavage to smaller proteins p24,p17,p9 and p6. The polymerase precursor protein is cleaved into products consisting of the reverse transcriptase (RT), the protease (PR) and the intergrase (IN) proteins[38]. Protease processes the gag and pol polyproteins and the intergrase is involved in virus integration into the cellular DNA.

Gene products of other spliced mRNA make up a variety of viral regulatory and accessory proteins that can affect HIV-1 replication in various cell types[38]. These regulatory proteins include Tat, a major protein involved in upregulating HIV-1 replication. Others are Rev, Nef, Vif, Vpr, Vpu and Vpx all of which appear to influence events such as assembly and budding of infectious viral particles[2,38]. Only two of the accessory gene products Tat and Rev are essential for the viral replication while the others appear to have auxiliary functions for viral propagation[38]. There are several ongoing studies on these regulatory proteins to try to determine their possible

HIV-1 GENOME AND VIRION STRUCTURE

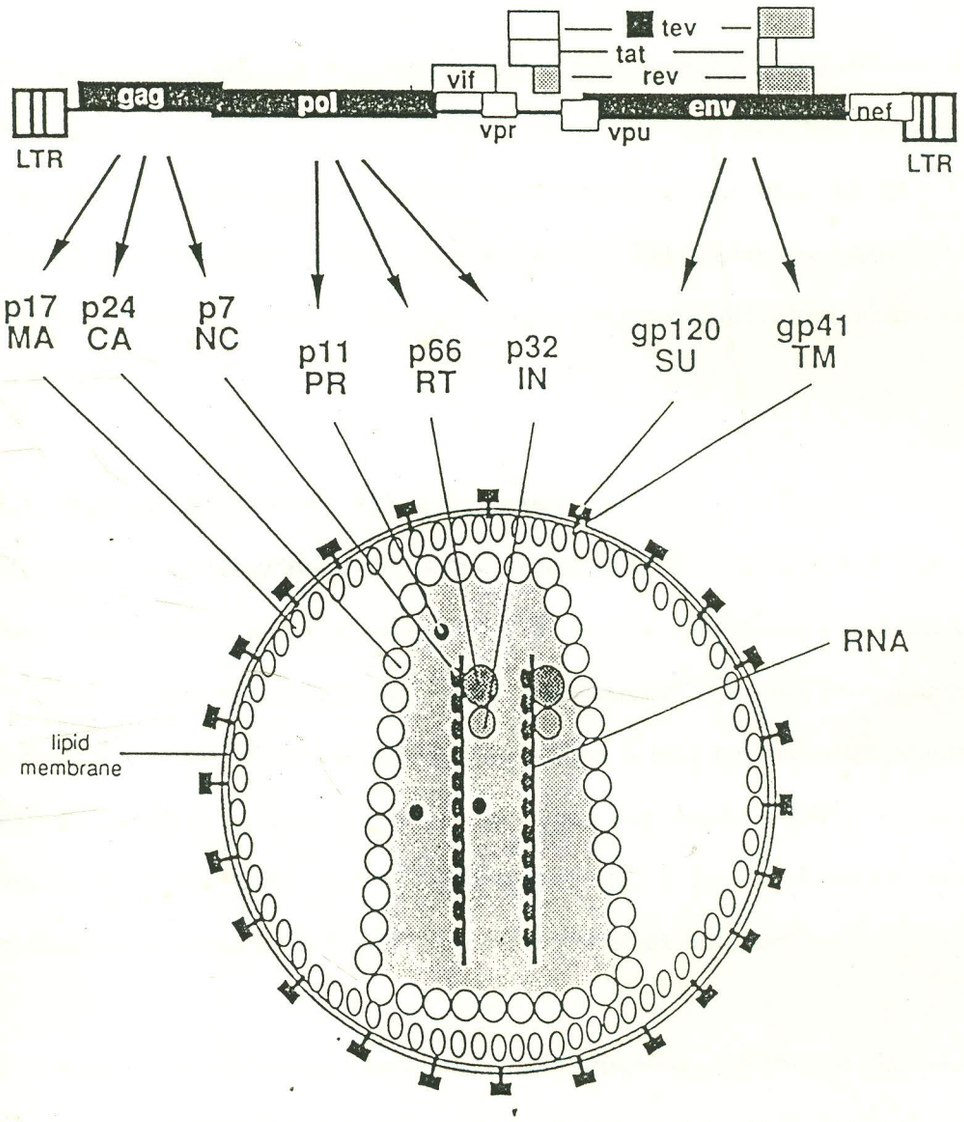


Figure 1. The structure of the HIV-1 genome and virion. The upper part shows the HIV-1 genome. Boxes indicate ORFs. The structural and enzymatic proteins produced by the gag, pol and env ORFs are indicated below the appropriate ORF. The locations of these proteins in the virion are indicated.

clinical application in the management of HIV-1 infected individuals. There are three enzymes that have been prime targets for antiviral approaches, they include the RNA-dependent DNA polymerase, intergrase and protease[2].

HIV-1 exhibits a striking degree of genomic heterogeneity that is evident when the nucleotide sequences of viral isolates from different infected individuals are compared[2]. This heterogeneity arises in part from the fact that the HIV-1 reverse transcriptase has a high replication error rate (1/1,700-1/4,000 nucleotides)[1,2]. Even distinct molecular clones from a single infected individual show sequence heterogeneity[2].

2.3. Natural history of HIV-1 infection:

Most of the knowledge about the natural history of HIV-1 infection has been obtained from crossectional and few prospective studies done in Europe and USA among intravenous drug addicts and homosexual populations[39-45]. Very few studies on the natural history of HIV infection have been done among heterosexual populations in Africa[46]. HIV-1 infects a variety of cells especially $CD4^+$ T-lymphocytes, macrophages and others such as trophoblastic cells, follicular dendritic cells, bowel epithelium, brain astrocytes, renal epithelium and many cell lines[2].

The time between infection with the virus and progression to clinical disease averages ten years or longer[40] but some individuals progress faster[47]. Following primary infection the virus rapidly replicates, an early burst of viraemia is often evident and a large number of viral particles spread throughout the body[47]. An estimated fifty to seventy five percent of individuals develop an acute viral syndrome within three to six weeks after initial infection. This is characterized by flu-like symptoms, maculopapular

rash, fever, myalgia, arthralgia, headache, diarrhoea, sore throat and neurological manifestations, but this may pass unnoticed[47]. Also during this period there is a large number of HIV-1 viral particles in the peripheral circulation and a significant drop in peripheral $CD4^+$ T-lymphocyte counts occurs[39,40,48]. Normally these symptoms last for one to three weeks, but accompanying lymphadenopathy, lethargy and malaise can persist for many months[39,40]. Within four to eight weeks of the infection an HIV-1 specific immune response is usually seen accompanied by dramatic decline in plasma viraemia[47]. The majority of patients then enter a clinical latency phase that may last for up to ten years or longer and during this period very few $CD4^+$ T-lymphocytes in the peripheral blood (1 in 1000 to 1 in 10,000) are infected with HIV-1 and viraemia is low or absent[40]. Ultimately the asymptomatic stage of HIV-1 infection is replaced by clinically apparent disease.

Extensive data on the rate of progression from HIV-1 infection to AIDS reveal the following rates; 0-2% at six years, 30-40% at eight years and 48% at ten years[39-43]. It is most probable that the natural history of HIV-1 infection in African patients may not be markedly different from that in North America and Europe. On the other hand, it is also possible that progression to AIDS phase may be faster in Africans due to various factors such as poor nutrition, high incidence of other infections and lack of good health care facilities to treat minor ailments. Natural history studies, many of which are in progress in Africa will be able to give a clearer answer to this question.

2.4. Immune response in HIV-1 infection:

The immune response against the HIV is one of the fields that has been well studied and which is still attracting a lot of scientific interest. After establishing itself in the body, HIV-1 initiates a very complex series of host-virus interactions. In many ways

HIV-1 is a very immunogenic virus and virtually all infected individuals develop antibody responses to several of the structural and non-structural protein products of the HIV-1 genome. The immune response is both humoral and cell mediated.

2.4.1. Humoral immune response:

A conventional response of the host against viral infection is the production of antibodies that attach to the virus and inactivate or neutralize it. Cytotoxic T-lymphocytes also play an important part as viruses are intracellular organisms. HIV-1 envelope is the major target for the humoral antibody responses. By comparing a variety of strains, conserved and divergent or variable regions of the virus have been identified especially in the outer membrane glycoprotein[49,50]. The epitopes believed to be primarily involved in antibody neutralization have been localized to the envelope gp120 and the external portion of gp41[49,50]. While anti-HIV-1 neutralizing antibodies against several laboratory strains have been demonstrated in most infected individuals, they don't have strong neutralizing activity against their homologous strains[51-53]. This observation could reflect escape from the humoral response and as the disease progresses neutralizing antibodies appear to be replaced by enhancing antibodies[54].

In general HIV-1 sera can neutralize HIV-1 but not HIV-2 strains but in contrast sera from HIV-2 infected individuals have been reported to cross-react and neutralize some HIV-1 strains in vitro[55] emphasizing reported similarities in the structure of the envelope of these two related viruses. Most studies on neutralizing sites on HIV-1 indicate that at least six regions of the viral envelope could be involved in HIV neutralization; five portions of gp120 and a region of gp41[2,49,50]. The clinical relevance of these neutralizing antibodies remains uncertain, they would have value when elicited by a vaccine but their effect in controlling viral spread following infection

is not clear. Therefore, the concept of neutralizing antibody remains an in vitro phenomenon as it is not clear whether it occurs in vivo. It is also probable that mutation of HIV-1 in an individual coupled with deceptive imprinting in the immune response to HIV-1 during the course of infection is responsible for failure of the body to clear the infection[56].

Another major humoral immune response is antibody mediated cellular cytotoxicity (ADCC). Antibodies of the IgG1 subclass to both gp120 and gp41 envelope protein have been shown to induce ADCC in several studies[57-59]. In this process, the antibody-antigen-coated cells are recognised by effector natural killer (NK) cells bearing Fc receptors or by other monocytes and killed by cytotoxic mechanism [59]. Only certain epitopes on HIV-1 envelope proteins induce this response because not all anti-envelope antibodies produce this activity[2]. The role of ADCC in controlling HIV-1 infection is not yet clear but in principle ADCC would seem most important soon after infection to kill incoming or post-entry virus-infected cells. Induction of ADCC response by vaccines would then be a valuable objective and this response might be helpful in preventing mother-to-child HIV transmission. ADCC could also accelerate disease progression via non infectious lympholysis which may occur when free viral proteins such as gp120 bind to cluster of differentiation (CD) molecules on uninfected lymphocytes making them susceptible to destruction via ADCC.

HIV-1 infected individuals have been found to have antibodies that can enhance viral infection either via complement or Fc receptors[54,60]. Nevertheless, there are conflicting reports on the role of the CD4 molecule mostly on T-lymphocytes in the enhancing process[2]. If the CD4 molecule is involved in antibody directed enhancement (ADE) then most investigators conclude that enhancement occurs because the virus-antibody

complexes are brought closer to the CD4 molecule after attachment to Fc or complement receptors[2]. Alternatively, if CD4 molecule is not involved, perhaps HIV-1 is brought to the cell surface via complement or Fc-receptor binding and then the virus fuses directly with the cell membrane[2,60]. The clinical importance of ADE is not known but its association with disease suggests that it has a role in pathogenesis.

2.4.2. Cell mediated immune responses to HIV-1 infection:

In most viral infections cell mediated immune response plays a vital role in arresting or eliminating the infectious agent. A major component of cellular immunity is the natural killer (NK) cell which recognizes and kills virus-infected cells in a non-MHC-directed manner[61,62]. In HIV-1 infection, this cell type has been found to have decreased function particularly as infected individuals progress to disease[1,2,61,62]. HIV-1 infected cells are killed by NK cells through the recognition of antibodies bound to the viral envelope proteins on the infected-cell surface[2].

Recent observations indicate that human $CD4^+$ T lymphocytes can be separated into two subsets T helper type-1 (T_H1) and type-2 (T_H2) based on the predominant cytokines they secrete when they are activated by an appropriate signal[63]. T_H1 cells secrete interleukin 2 (IL-2) and interferon gamma predominantly, while T_H2 cells secrete IL-4, IL-6 and IL-10[63]. From studies of HIV-1 infected individuals a hypothesis has been presented that the T_H1 and T_H2 cytokines play an immuno-regulatory role in HIV-1 infection and can affect progression to AIDS[64]. T_H1 cell responses are found primarily in healthy asymptomatic individuals and in high-risk individuals who do not show evidence of HIV-1 infection[64]. A subsequent T_H2 response would lead to B-cell activation and hypergammaglobulinemia a well documented finding in HIV-1 infected individuals probably secondary to the IL-4 and

IL-6 production by the T_H2 cells. Since the T_H1 cells produce IL-2 and other cytokines that help in enhanced $CD8^+$ T-lymphocyte activity, this subset could also be very important in the cellular immunologic control of HIV-1 infection and prevention of AIDS[64].

Cytotoxic $CD8^+$ T-lymphocytes (CTL) are able to recognize virus-infected cells in association with major histocompatibility antigen class one (MHC-1)molecules. Studies on the antiviral response has shown that $CD8^+$ T-lymphocytes can kill cells expressing several different HIV-1 proteins including reverse transcriptase, envelope, core and some accessory proteins[65-67]. The cytotoxic $CD8^+$ T-lymphocytes can be found in relatively large numbers during the asymptomatic period but then they appear to decline in numbers and at least in the antiviral activity in some individuals with progression of disease[2,67]. Although this type of antiviral activity has prevented virus spread in some animal model systems[68] the role of CTL in HIV-1 infection in man is not clear. Despite some correlation of CTL activity with a healthy clinical state[59] progression of disease with increasing levels of HIV-1 infected cells occurs in the presence of these $CD8^+$ T-lymphocytes[2]. Nevertheless, CTL are known to limit viral spread by lysing infected cells and secreting an unidentifiable protein which suppresses viral replication[2]. Cytotoxic T-lymphocytes also secrete interferon gamma and tumour necrosis factor (TNF) which are known to upregulate HIV-1 genes in resting cells[2].

2.5. Immunopathology of HIV-1 infection:

The central immunopathophysiologic feature of HIV-1 infection is the loss of $CD4^+$ T-lymphocytes which become detectable about the time of seroconversion in some individuals[69]. It is also well established that AIDS is a consequence of depletion of this very important cell in the immune system[1,2,61,69]. The degree of loss of $CD4^+$ T-

lymphocytes remains an excellent predictor of the time to onset of frank AIDS[40]. The mechanism by which HIV-1 causes loss of $CD4^+$ T-lymphocytes is an area of controversy. Several studies suggest that immune abnormalities can be observed in T-, B-lymphocytes and macrophages early in infection even before the loss of $CD4^+$ T-lymphocytes begins[61,65]. In efforts to explain the mechanisms of HIV-1 induced cell killing and thus immunodeficiency several options have been suggested including:

1. Accumulation of viral proteins:

Several studies have associated cell death with direct toxicity from the accumulated viral particles or virus envelope proteins[61]. The relative quantities of the viral envelope proteins produced by the cell can determine cytopathicity by interfering with the metabolism of the cell. Moreover viral proteins cause disturbance in membrane permeability resulting in loss of intracellular ionic strength[2,61].

2. Apoptosis:

Apoptosis is a recently described phenomenon which involves the in-appropriate re-emergence of a programmed T-lymphocyte loss that is a normal physiological response during thymocyte maturation[2]. During ontogeny T-lymphocytes recognizing any self proteins in association with self MHC are positively selected in the thymus gland and killed through apoptotic mechanism. In vitro it has been observed that attachment of gp120 to $CD4^+$ T-lymphocytes followed by cross linking with antibody to gp120 or stimulation of cell using anti-CD3 lead to this programmed cell death[2]. Therefore, envelope protein expressed on the surface of HIV-infected cells may prime neighbouring uninfected cells for apoptosis by cross-linking the CD4 molecule and subsequently by activation with antigen. The process is known to require cell activation, protein synthesis

and the action of Ca^{++} dependent endogeneous endonuclease that produces fragmentation of the cellular DNA into small measurable nucleotide units. Apparently $CD4^+$ T-lymphocytes do not undergo apoptosis in HIV-1 infected chimpanzees[61,67], probably explaining lack of disease in these animals. Apoptosis is therefore now thought to be one of the most important mechanism accounting for fall in the number of $CD4^+$ T-lymphocytes in HIV-1 infected individuals[1,2]. It has also been shown that $CD8^+$ as well as $CD4^+$ T-lymphocytes from HIV infected individuals undergo apoptosis in vitro[1,2].

3. Accumulation of HIV-1 in infected cells can affect the function, long-term viability and replication of $CD4^+$ T-lymphocytes. It can also interfere with the normal events and signal transduction[2]. Direct killing of non-infected $CD4^+$ T- lymphocytes coated with free envelope glycoproteins by cytotoxic T-lymphocytes althrough ADCC is also thought to contribute to t he depletion of $CD4^+$ T -lymphocytes seen in HIV-1 infection[58-60].

4. Other possible mechanisms include the effect of viral proteins and immune complexes, syncytia formation, cytotoxic cells and yet unidentified $CD8^+$ T- lymphocyte suppressor factors, anti-lymphocyte antibodies and effects of cytokines[61,65]. HIV-1 infected cells can also undergo changes in their MHC class II phenotype and become susceptible to autoimmune attack probably explaining the autoimmune-like conditions which are seen in HIV-1 infected individuals[65].

Therefore, the HIV-1 infects two cell types that are involved in virtually all immune responses, the $CD4^+$ T-lymphocytes and cells of the monocyte-macrophage lineage which are important antigen presenting cells[69]. The tropism of HIV-1 to these cells is due to the high affinity of the HIV-1 gp120 for the cell surface glycoprotein CD4 which is expressed in high levels on cells of the $CD4^+$ T-lymphocyte subset and at a lower level on monocytes and macrophages[69]. The immunologic abnormalities in HIV-1 infected individuals must be viewed in the context of the on-going immune response to HIV-1. In infected individuals, immunodeficiency develops despite the presence of readily detectable B-and T-lymphocyte response to HIV-1.

With respect to humoral immune response, levels of neutralizing antibodies are low even when high levels of antibodies to HIV-1 envelope glycoprotein are present[66]. Polyclonal B-lymphocytes activation with concomitant hypergamma-globulinemia is a consistent feature of the clinical picture of HIV-1 infection[61,66]. With regard to CTL response, HIV-1 specific $CD8^+$ CTL are readily detected in healthy seropositive individuals and are sometimes detected in patients with AIDS[2,67].

2.6. Prognostic markers in HIV-1 infection:

Identification of clinical and laboratory parameters that will predict progression to AIDS in people infected with human immunodeficiency virus is important for clinical management, counselling of patients and for evaluation of therapeutic intervention(s). A number of markers of disease progression have been identified including clinical features, immunologic and virologic laboratory parameters. Evaluation of some of these parameters have been found to correlate with the risk of progression to AIDS and others are able to predict survival. Using AIDS as a surrogate end point of evaluating these markers is hampered by the heterogenous definition of AIDS and the long

incubation period of HIV-1 infection making it difficult to identify early markers for disease progression.

2.6.1. Clinical Markers:

In the natural history of HIV-1 infection there are a number of clinical conditions which have been evaluated as possible predictive markers. Early reports suggested that disappearance of generalized lymphadenopathy was a significant marker of disease progression[70]. However, studies done later showed that this syndrome does not predict subsequent progression to AIDS[71,72]. HIV-1 infected patients with oral candidiasis (thrush) have been found to have a significantly increased risk of progressing to AIDS[73]. There are conflicting reports regarding the predictive power of herpes zoster. In one study an episode of herpes zoster appeared to herald a more rapid occurrence of AIDS[40]. In another study herpes zoster was diagnosed two to seven years after HIV-1 infection but lacked predictive power[74] and the two-year progression rate to clinical AIDS for men with herpes zoster was 23%. Oral hairy leucoplakia (OHL) is an infection of the tongue or oral cavity with Epstein-Barr virus and requires an expert eye for diagnosis. In one cohort, oral thrush and hairy leucoplakia were diagnosed on average about a year later than herpes zoster and were shown to be of limited value as markers of disease progression[75]. Constitutional symptoms such as weight loss, fatigue and persistent diarrhoea are relatively late manifestations but are highly specific for imminent progression to AIDS and are unlikely to occur until three to five years after infection[40]. Therefore, it can be seen that currently there is no good clinical marker of disease progression or one which can be used in intervention studies.

2.6.2. Laboratory Markers:

Failure to identify promising clinical prognostic markers has prompted researchers working on HIV infection to look for laboratory markers of disease progression. Currently there are a number of markers which have shown promising results and have been well reviewed[41]. These are broadly divided into viral markers which are a product of replication of HIV-1 in the body sometimes referred to as direct or specific markers and immunological markers which are the result of the immune response to HIV-1 infection also referred to as indirect or non-specific markers.

2.6.2.1. Viral markers:

A direct measure of viral load is required for optimal monitoring of HIV-1 infected individuals. Such monitoring could then delineate the natural history of the infection as well as to assess the effectiveness of new antiretroviral therapies. Viral p24 antigen level and viral load are the two common viral markers which have been studied extensively. Viral load has three major components; cell-free virus in plasma, infected cells containing both actively replicating virus and viral components and cells containing proviral DNA. The existence of all these compartments possibly distributed throughout the body, makes this measurement quite difficult. One practical approach is to assume that the viral load in the peripheral blood will reflect the viral load of the entire body. Recent studies show that one out of ten $CD4^+$ T-lymphocytes in AIDS patients and one out of a thousand $CD4^+$ T-lymphocytes in some asymptomatic individuals have detectable viral particles[48]. Therefore determination of viral particles in these cells either by viral culture or by polymerase chain reaction (PCR) can represent a meaningful estimate of viral load bearing in mind the distribution of such cells in peripheral blood as compared to the rest of the lymphoreticular system.

2.6.2.1.1. Viral p24 antigen:

In the course of HIV-1 infection viral p24 antigen is released to the circulation followed by formation of antibodies against the protein. The antibody response is so intense that large amounts of antibody against the virus form immune complexes which are not detected by the conventional assays[76]. As a result, detectable p24 antigen levels are found mainly during early and late stages of HIV-1 infection but in only a small proportion of infected individuals[2]. Nevertheless, there have recently been great improvements in p24 antigen detection assays by employing various techniques of immunecomplex dissociation[77,78]. In Europe and North America it has been shown that ten to twenty percent of asymptomatic HIV-1 infected homosexual men and haemophiliacs will have detectable HIV-1 p24 core antigen by ELISA[76-78]. Several studies have shown the predictive power of p24 antigen mostly when combined with other markers like $CD4^+$ T-lymphocytes counts and B2-M levels[79-82]. However studies in Africa have shown that even a much smaller proportion of patients infected with HIV-1 have detectable levels of p24 antigen thus limiting the usefulness of this antigen test in this population[83,84]. In these studies those patients who appeared to be p24 antigen positive at seroconversion were shown to have a more rapid loss of $CD4^+$ T-lymphocytes and thus a more rapid progression to AIDS[83,84]. The lack of utility of antigen detection in monitoring disease progression appears to be related to the relative insensitivity of the test so that high levels of antigen are needed for a positive test result. Such high levels are found at late stages of the disease and at this time $CD4^+$ T-lymphocyte counts and other prognostic indicators are also markedly abnormal.

2.6.2.1.2: Viral load:

Crossectional studies have suggested that viral burden in an HIV-1 infected host increases over time[2]. Thus laboratory quantitation of the amount of HIV-1 present

might be expected to be a useful prognostic indicator. The amount of virus present in infected individuals can also be assessed by quantitative culture of plasma and this has been found to correlate with stage of the disease[85]. However, the technique of quantitative culture is expensive, time consuming and results are available after a week or so. Evaluation and quantitation of HIV-1 DNA using PCR technology has recently been developed and shows promising results in monitoring prognosis of HIV-1 disease[86]. A recent report has also demonstrated that the number of copies of HIV-1 DNA in peripheral blood of HIV-infected persons increases with advancing disease[87]. While the technique is not well established yet, it seems that RNA PCR method for viral quantitation will make viral load determination the best marker of disease progression and for evaluation of antiviral measures[86,87]. A major advantage of viral load as compared to other viral markers is that, the former is a true measure of viral activity despite the fact that most of the viral multiplication and localization is in the lymphoid organs[2].

2.6.2.2. Immunological markers:

Immunological markers of disease progression are sometimes called indirect markers because they are a reflection of the body's reaction to HIV-1 infection unlike the direct viral markers discussed above. They include lymphocyte subpopulation determinations and estimation of concentration of serum immune activation markers. These techniques are relatively simple, the methodologies for their determination are well established and results can be obtained quickly.

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2.6.2.2.1. Lymphocyte subpopulation:

Lymphocytes are broadly grouped into thymus derived lymphocytes (T-lymphocytes), bone marrow (bursa equivalents) derived lymphocytes (B-lymphocytes) and so called null cells. The T-lymphocytes are further classified into two major functional groups namely the helper/inducer and cytotoxic T-lymphocytes. The helper/inducer cells are characterized by having the cluster of differentiation 4 molecule ($CD4^+$)while the cytotoxic cells have the CD8 molecule ($CD8^+$). The cluster of differentiation molecules are membrane surface markers on all leukocytes which are identified using specific monoclonal antibodies[88]. The CD4 molecule has been shown to be the receptor for HIV[69]. It has also been well established that HIV-1 produces slow and predictable attrition of the $CD4^+$ T-lymphocyte counts from the time of seroconversion[69]. In the early asymptomatic phase $CD4^+$ T-lymphocyte counts may remain stable and within quoted adult reference ranges for long periods of time[49,50]. A small and diminishing proportion of adults show normal $CD4^+$ T-lymphocyte counts five years or more after documented HIV-1 infection[89]. Most published cohorts however indicate that $CD4^+$ T-lymphocyte counts fall progressively in most infected patients and this trend can be established virtually in all patients by sequential monitoring[37,45,90,91]. The rate of fall of $CD4^+$ T-lymphocytes is faster in some patients than in others with age as a clear influencing factor with older and very young patients progressing more rapidly[45] probably reflecting the functional capacity of the immune system in these two extremes of life.

Studies done in Europe and North America on homosexual and intravenous drug users show a steady decline in proportion and absolute number of peripheral $CD4^+$ T-lymphocytes[80,92-96]. The absolute counts fall from a reference level of 800-900 cells per microliter (ul) by an average of 60-100 cells per year[92-96]. Many of these studies

show clearly that $CD4^+$ T-lymphocyte counts can predict progression to AIDS. Few such studies have been done in Africa[97,98]. While percentage $CD4^+$ T-lymphocytes, $CD4^+$ T-lymphocyte counts and CD4:CD8 ratio have excellent prognostic value for predicting the development of AIDS and are highly correlated to each other, the $CD4^+$ T-lymphocyte percent and the CD4:CD8 ratio are slightly better predictors of time to development of AIDS than the absolute $CD4^+$ T-lymphocyte number[92].

Lymphocyte subsets determination is usually done using flow cytometry[99] which requires a big capital investment, well trained manpower and precise quality control[99-102]. Presently, very few techniques are available to measure $CD4^+$ T-lymphocyte counts directly. The $CD4^+$ T-lymphocyte counts as measured by flow cytometry is a product of the peripheral white blood cell counts (WBC), the differential fraction of lymphocytes measured by manual differential leucocyte counting or by automated methods and the percentage of the $CD4^+$ T-lymphocytes from the flow cytometer. Therefore fluctuation in any of these measurements could result in variability in the calculated $CD4^+$ T-lymphocyte counts[99,103]. It is important to note that circulating lymphocytes represent only a fraction of the total lymphocytes in the body with the rest being found in the primary and secondary lymphoid organs[104].

Several investigators have documented diurnal rhythm in $CD4^+$ T-lymphocyte counts in healthy HIV-1 antibody negative adults[105]. In these adults the $CD4^+$ T-lymphocyte counts is lowest in the morning (08.00 - 11.00 hours) and highest in the evening (22.00 hours) with the evening $CD4^+$ T-lymphocyte counts being double the $CD4^+$ T-lymphocyte counts of the morning nadir[105]. Factors like seasonal variation, stress, exercise, exogenous glucocorticoids, serum cortisol and acute or chronic viral infections have been reported to affect $CD4^+$ T-lymphocyte counts[106]. Racial

differences in haematological parameters makes it necessary to establish reference $CD4^+$ T-lymphocyte values in each population[98,107,108]. Decrease in the levels of $CD4^+$ T-lymphocytes in HIV-1 infection is not constant and there are reports of lymphopenia without evidence of HIV infection[109] and also of HIV-1 infected individuals whose $CD4^+$ T-lymphocyte counts remain stable for a prolonged period of time, the so called non-progressors[89]. The phenomenon of non-progression to AIDS is an area of intense study in order to identify specific factor(s) in these individuals and probably enhance such factor(s) in order to halt progression of the disease. Recently simpler methods of lymphocyte subset determination have become available and their evaluation so far seem to indicate promising results[110-112].

2.6.2.2.2: Immune activation markers:

Human immunodeficiency virus type-1 is a potent immunogen probably due to the outer glycoprotein on the surface. Immune stimulation has been shown to play a major role in immunopathology due to HIV-1 infection as shown by several pathological conditions which have been recognized[1,2,65]. Several immune activation markers have been shown to have prognostic power among HIV infected individuals either alone or in combination with lymphocyte subsets determination[81,113-115]. Some of these activation markers include beta-2 microglobulin (B-2M), neopterin, soluble CD8, soluble CD30 and interleukin (IL) 2 among several others.

2.6.2.2.2.1. Beta 2 microglobulin:

Beta-2 microglobulin is the most widely studied soluble immune activation marker in HIV-1 infected individuals. It is a 11,800-dalton short polypeptide that appears to be a premodial precursor of the immunoglobulin domain and is coded for on chromosome 15 in humans (q21-22)[116]. B-2M antigen is located extracellularly in the human

histocompatibility complex (MHC) (HL-A, B and C) and it serves as light or non-specific structural chain of class I antigen of the MHC[116]. B-2M is thus present on the surface of most nucleated cells but in greatest density on lymphoid cells and especially T-lymphocytes[117]. Its function is unknown but there is some recent evidence suggesting a role in presenting antigens to $CD8^+$ T-lymphocytes through its interaction with the antigen specific MHC class-I chain and it is believed to reflect lymphocyte turn over, thus a marker of immune activation[118].

The immune status reflected by determination of B-2M may be preferable to evaluation based on peripheral blood cells because serum B-2M reflects activity in all lymphoid tissues while peripheral lymphocytes are just a fraction of the whole pool of lymphocytes[104]. Serum B-2M levels are often elevated in inflammatory disorders such as rheumatoid arthritis, systemic lupus erythematosus (SLE), Sjogrens syndrome and Crohns disease. Levels are also elevated in lymphoproliferative disorders including multiple myeloma, B-cell lymphoma and chronic lymphocytic leukaemia[119-120]. Serum B-2M can be estimated in serum by using radio immunoassay or by an ELISA, the latter making it a suitable test in small laboratories with limited facilities. Serum B-2M predicts progression to AIDS both independently of and in addition to $CD4^+$ T-lymphocyte counts[95,96,121-123]. Beta 2 microglobulin rises from a normal value of 1.7 mg/l in HIV-1 uninfected individuals to values of 4-5 mg/l just before AIDS[121-123]. In studies which have been done in Tanzania and neighbouring Rwanda, elevated B-2M were associated with higher risk to early death among HIV-1 infected patients[121,122].

2.6.2.2.2.2. Soluble CD8:

In the natural history of HIV-1 infection a steady increase of $CD8^+$ T-lymphocyte cell population as the disease progresses has been reported [124]. Moreover, soluble CD8 (sCD8) molecules are released from activated peripheral blood mononuclear cells in vitro[125] as well as from $CD8^+$ human leukaemic cell lines[126]. Soluble CD8 is a 52kD homodimer, which is 5kD smaller than its membrane-bound counterpart and the release of sCD8 apparently is not due to destruction, but rather to immune stimulation especially in HIV-1 infection[113-115]. In vivo, sCD8 levels are elevated in patients with $CD8^+$ T-lymphocyte leukemias and various paediatric lymphomas, as well as in patients with acute Epstein-Barr virus (EBV) -induced mononucleosis[127,128]. Also changes in levels of sCD8 have been found to parallel the change in the number of CD8/HLA-DR cells which are markers of activated cells[126]. Elevated levels of sCD8 in sera of HIV-1 infected populations have been reported[125,128,130] though it was not conclusive whether it had any prognostic value. Bearing in mind the probable central role of CTL in non progressors, levels of soluble CD8 may prove to be a good prognostic marker in the near future[89].

2.6.2.2.2.3. Neopterin:

Neopterin is produced in activated macrophages from guanosine triphosphate (GTP) during the biosynthesis of tetrahydro-biopterin [131]. Gamma interferon has been shown to be an excellent neopterin inducer as it activates macrophages[115]. Urinary and serum neopterin levels increase concurrently with the appearance of malignancies[131]. Serum neopterin is increased in HIV-1 infected individuals when compared with healthy heterosexuals, uninfected homosexual men or uninfected intravenous drug users and its level increases as HIV-1 infection progresses[113,131]. It

has been shown that determination of neopterin levels adds to the predictive value of $CD4^+$ T-lymphocyte counts and other immune activation markers such as B-2M[131]. In the laboratory neopterin is usually estimated using radio immunoassay.

2.6.2.2.2.4 Soluble CD30:

Soluble CD30 (sCD30) is a marker of immune activation which is expressed mainly in a few activated T-lymphocytes and B-lymphoblasts within the normal lymphoid tissue[132]. CD30 is a 120 kd surface antigen originally identified by the Ki-1 monoclonal antibody developed against Hodgkin's and Reed-Steinberg cells [133]. A soluble form of CD30 (sCD30) has been shown to be released by $CD30^+$ cells in vitro and in vivo[134]. Increased serum levels of sCD30 have been described in patients with $CD30^+$ lymphomas as a by product of neoplastic cells[134]. Recently, levels of sCD30 have been shown to be an early independent predictor of progression to AIDS[135].

3.0. Rationale of the study.

The first patients with HIV-1 infection in Tanzania were identified in 1983 and more than ten years later there is no clear indication yet that the epidemic is under control[24,29]. Moreover, there is no effective treatment or vaccine against the infection. A clear understanding of the natural history of HIV infection is therefore important in order to identify important factors in controlling progression to AIDS among people who are infected. Previous reports show that there are both racial and sex differences in the reference values of various laboratory parameters including lymphocyte subsets[99,102,108,112]. Therefore there is always a need to establish reference values and also study the variation of these values with time in Tanzania since this has not be done.

Several clinical and laboratory prognostic markers have been found to have ranging predictive powers of progression to AIDS and survival after infection[41,80]. Several of these studies have been done on both crossectional study design and prospective cohorts in Europe and North America on haemophiliacs, homosexual men and on intravenous drug users. Few such studies can be cited in Sub Saharan Africa which has more than 70% of all HIV-1 infected individuals in the world[97,98,121]. Among the laboratory markers of disease progression, $CD4^+$ T-lymphocyte counts and beta-2 microglobulin have been found to be good prognostic markers. Several biological parameters are known to vary with time in healthy persons and this variation has to be taken in consideration when the particular parameter is measured in infected individuals. It is hypothesized that the normal reference values of lymphocyte subsets and B-2M in our population will not be significantly different between sexes and from other ethnic groups and that these parameters will be found to be good prognostic markers in HIV infected individuals. The present studies therefore aimed at

investigating the variability of these prognostic markers in healthy HIV seronegative adults and to evaluate the performance of the markers as determinants of disease progression in HIV-1 infected Tanzanian adults.

4.0. Objectives:

4.1. Broad objective:

To study immunological markers of disease progression in human immunodeficiency virus infected adults in Dar es Salaam, Tanzania.

4.2. Specific objectives:

1. To study the variability of lymphocyte subsets and beta-2 microglobulin in a cohort of HIV seronegative individuals.

2. To establish the reference values of lymphocyte subsets and beta-2 microglobulin among HIV seronegative adults.

3. To determine the levels of B-2M, T-lymphocyte subsets and sCD30 in HIV infected adults at different clinical stages.

5.0. SUBJECTS AND METHODS.

5.1. Study 1: Variability of counts of peripheral leucocytes, lymphocyte subsets and of Beta-2 microglobulin in HIV-1 seronegative male and female adults in four years of followup.

The study subjects were HIV-1 seronegative individuals who had been selected as referents in an on going study on the natural history of HIV infection in Dar es Salaam. The study base consisted of an open cohort of workers in three hotels who have been followed since 1990 when they were invited for an annual medical examination. By using a structured questionnaire (see appendix), the study group was interviewed and underwent physical examination. Verbal informed consent for testing blood for various infective conditions including HIV infection was solicited from each individual at each visit. Verbal informed consent was used because not all clients were able to read and write. The study individuals were seen at their respective working places once per year where they underwent physical and laboratory examination. Screening for HIV antibodies was done on each visit.

Two blood samples one in sterile ethylene diamine tetra-acetic acid (EDTA) vacutainer and another in a sterile vacutainer tube were obtained from each client during the follow-up visits. The anticoagulated blood sample was collected between 8.00 hr and 12.00 hr, mixed immediately and kept at room temperature before testing. All sera were screened for antibodies to HIV using Enzygnost anti-HIV-1+2 (Behring, Marburg, Germany) ELISA and all reactive sera were further tested by a Western blot assay (Dupont Hercules, CA; U.S.A.). The WHO criteria for interpretation of HIV-1 and HIV-2 Western blots was adopted[136]. Peripheral total white cell counts and

haemoglobin were determined using a Coulter counter (Coulter Electronics, North West Drive Luton, London). Determination of T-lymphocyte subsets was done on whole blood within six hours of collection by staining with the following monoclonal antibodies conjugated to either phycoerythrin (PE) or fluorescein isothiocynate (FITC): Simulset LeucoGate (anti-HLe-1/CD45, anti-Leu M3/CD14), Isotype control (IgG1+ IgG2a),

anti-Leu2a/CD8, antiLeu3/CD4 and anti-Leu4/CD3 (Becton Dickinson, Immunocytometry System, CA. USA) (BD). The stained samples were analyzed by a fluorescent activated cell counter (FACScan)(BD) after fixation with 0.5% formaldehyde solution. Beta-2 microglobulin levels in serum were estimated using Pharmacia Beta-2 EIA (Pharmacia Diagnostics, Uppsala, Sweden) according to manufacturers recommendations. Levels of B-2M were interpolated from a standard curve obtained by using calibrators which were run together with the test serum samples.

5.2. Study 2: Immunohaematological findings including lymphocyte subsets and beta-2 microglobulin levels in HIV-1 infected adults at different clinical stages .

The study was based on both a crossectional and a longitudinal population groups in Dar es Salaam. In the crossectional study, the study population consisted of HIV-1 antibody negative healthy blood donors and HIV-1 infected patients who were either admitted to medical wards or who were seen as out patients at the Muhimbili Medical Centre (MMC). The patients were examined and staged based on the WHO proposed staging system for HIV infection and disease[137]. After verbal informed consent (or as part of routine investigation), venous blood was taken from each individual before midday. In the longitudinal study, the study base consisted of an open cohort of

workers in three hotels who have been followed annually for four years. The study population was interviewed using a structured questionnaire followed by general physical examination. Verbal informed consent for testing blood for various infective conditions including HIV infection was solicited from each individual. For every HIV-1 antibody positive individual entered in the study, two HIV-1 seronegative referents matched for age and sex were recruited.The study population was seen every twelve months at their respective working places where they were examined. The referents constituted the study sample for study 1 referred to above.

Testing for HIV antibodies was done on each participant every year. All the HIV-1 antibody infected individuals in the study were staged using the proposed World Health Organisation (WHO) HIV clinical staging criteria[20]. During follow up verbal information was obtained from the employer and friends regarding health status of those who were absent from their working places due to illness. The blood samples were taken and levels of WBC, lymphocyte subsets and B-2M were determined as in study one above. Soluble CD30 (sCD30) concentration was estimated in the sera of individuals whose dates of seroconversion were known using DAKO CD30 (Ki-1 Antigen) ELISA (DAKO Glostrup, Denmark). B-2M and sCD3O levels were interpolated from standard curves obtained from provided standards which were also run with test sera.

5.3. Statistical analysis.

Lymphocytes subsets and concentrations of B-2M were skewed so the results were analysed using non parametric tests namely Kruskal-Wallis one way analysis of variance by ranks, Mann-Whitley U test and Spearman rank correlation coefficient test. A two-tailed p value less than 0.05 was considered significant.

6.0. RESULTS.

6.1. Study 1.

After a followup of 4 years, 416 clients who were HIV-1 antibody negative had been seen. They included 313 (75.2% males and 103 (24.8%) females with mean ages of 33.5 (20.2, 46.4 95% CI) and 28.3 (17.9, 38.6 95% CI) years for males and females respectively. The sex difference in age was statistically significant (p= 0.0001). The number of clients seen in relation to the number of visits made is shown in Table 1. Two hundred forty two (72.3%) males and 81 (78.6%) females were seen more than once in the period of 4 years. When one of the hotels was closed at the end of 1993 all the study population from that hotel were lost from the followup .

Table 2 shows the sex differences in the various parameters studied. Females had significantly higher percentage $CD3^+$ and $CD4^+$ T-lymphocyte counts and CD4:CD8 ratio, but they had significantly lower $CD8^+$ T-lymphocyte counts and B-2M levels. When controlled for sex age differences there was still significant differences in B-2M concentrations, percent $CD4^+$ and $CD8^+$ T-lymphocytes counts between the two sexes ($p<0.0025$). Generally the percent lymphocytes obtained in both males and females were lower in 1994 as compared with findings in the previous years probably due to technical change in the method of lysing and aspiration of samples such that more lymphocytes could have been lost. However, the lower mean concentration of B-2M obtained in 1992 and 1994 could not be due a technical problem because all samples obtained from 1991 to 1993 were done together. Considering workers who were seen for four years consecutively, in males total lymphocyte, percent $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes and concentrations of B-2M were significantly different in the four years of follow up ($p<0.005$) (Table 3). During 1993, the Coulter counter was defective hence no counts of WBC and lymphocytes were available. However, total

WBC counts, percent $CD4^+$ and $CD8^+$ T-lymphocytes were not significantly different. In females, total lymphocytes and $CD3^+$ T lymphocytes were the only parameters which were significantly different during the follow up period (Table 4). In both sexes the percent $CD4^+$ and $CD8^+$ T-lymphocytes were more reproducable than absolute counts and there was no correlation between absolute $CD4^+$ and $CD8^+$ T-lymphocyte counts and their respective percentages.

Table 1. Number of visits made by the study population.

	MALES		FEMALES	
No.of visits	**no**	**%**	**no**	**%**
1	71	22.7	22	21.4
2	108	34.5	28	27.2
3	69	22.0	28	27.2
4	65	20.8	25	24.3
Total	**313**	**100**	**103**	**100**

Table 2. Sex differences in the median and geometric mean values [95% confidence interval (reference range)] of WBC, lymphocyte subsets and beta-2 microglobulin in HIV-1 seronegatives.

	MALES	FEMALES	
Parameter	n=210 median mean	n=70 median mean	p. value
WBCX 10^9/L	5.7 5.8 (3.0, 11.9)	5.5 5.5 (3.1, 9.5)	0.2405
Lymph %	37.0 36.9 (22.4, 60.6)	37.0 36.5 (23.6, 56.3)	0.8484
Lymph/ul	2160 2131 (1025, 4430)	1885 1993 (1012, 3923)	0.1529
CD3+ T %	70.0 67.7 (49.1, 93.2)	72 72.4 (58.1, 90.3)	0.0039
CD4+ T %	38.0 36.3 (23.6, 56.6)	44.0 43.3 (31.8, 58.9)	0.0001
CD4+T/ul	785 786 (568, 1088)	840 863 (347, 2151)	0.1295
CD8+T%	27.0 27.0 (14.2, 51.6)	27.0 25.5 (16.5, 39.4)	0.089
CD8+T/ul	570 576 (230, 1446)	490 490 (224, 1076)	0.0109
CD4:CD8 RATIO	1.37 1.35 (0.58, 3.13)	1.69 1.75 (0.96, 2.99)	0.0001
B-2M g/l	1.7 1.6 (0.73, 3.54)	1.3 1.3 (0.65, 2.61)	0.0001

Table 3. Variability of median and geometric means of WBC, lymphocyte subsets and beta-2 microglobulin among HIV-1 seronegative males during a four year period of follow up.

		YEARS OF FOLLOW UP				
		1991	1992	1993*	1994	
Parameter	n	median mean	median mean	median mean	median mean	p value
WBCX 10^9/L	66	6.2 6.1	5.2 6.0	- -	6.0 6.1	0.08855
Lymph %	66	38 36.3	38 37.7	37 36.7	32 31.5	0.0001
Lymph/ul	66	2160 2195	2180 2229	- -	1950 1909	0.0039
CD3+ T %	66	67.5 66.4	69 67.2	67 66.6	64 64.2	0.0055
CD4+ T %	66	36 36.8	37 36.9	38 37.3	37 36.3	0.8602
CD4+T/ul	66	820 819	840 824	- -	710 696	0.0003
CD8+T%	66	26 25.9	26 26.1	25 25.2	27 25.7	0.0203
CD8+T/ul	66	430 578	487 583	- -	490 487	0.0058
CD4:CD8 RATIO	66	1.39 1.42	1.46 1.41	1.50 1.48	1.41 1.41	0.0692
B-2M g/l	45	1.6 1.5	1.6 1.5	1.9 1.8	1.4 1.4	0.0007

*No WBC and lymphocyte counts were available during 1993 due to breakdown of the Coulter counter

Table 4. Variability in median and geometric means of WBC, lymphocyte subsets and beta-2 microglobulin in HIV-1 seronegative females during a four year period follow up.

		YEARS OF FOLLOW UP				
		1991	1992	1993*	1994	
Parameter	n	median mean	median mean	median mean	median mean	p value
WBCX 10^9/L	27	5.2 5.3	5.4 5.2	- -	6.0 5.6	0.7485
Lymph %	27	34 34.0	33 33.8	34 33.2	29 29.1	0.0247
Lymph/ul	27	1850 1796	1767 1760	- -	1630 1648	0.6412
CD3+ T %	27	74 72.2	72 72.3	73 72.7	72 70.4	0.0466
CD4+ T %	27	46 43.8	46 43.4	48 44.6	44 44.3	0.149
CD4+T/ul	27	820 785	800 837	- -	740 735	0.3679
CD8+T%	27	27 25.3	25 24.5	28 24.1	25 23.5	0.1407
CD8+T/ul	27	430 454	430 430	- -	360 387	0.0719
CD4:CD8 RATIO	27	1.61 1.72	1.74 1.77	1.92 1.84	1.75 1.89	0.0657
B-2M g/l	20	1.4 1.3	1.3 1.3	1.5 1.5	1.2 1.1	0.411

*No WBC and lymphocyte counts were available during 1993 due to breakdown of the Coulter counter

6.2. STUDY 2

A total of 119 HIV-1 antibody negative blood donors and 183 HIV-1 antibody positive patients in different stages of HIV infection were studied in the crossectional study. The mean ages were 28.8 (95% C.I 27.4-30.3) and 33.5 (95% C.I 32.0, 35.0) years for blood donors and patients respectively and these were statistically different. Among the blood donors 68 (57.1%) were males while 51(42.9%) were females. Ninety nine (54.0%) of the patients were males while 84 (46%) were females. The distribution of the patients in stages one, two, three and four of HIV infection based on WHO staging criteria was 52 (28.3%), 50 (27.4%), 41 (22.5%) and 40 (21.6%) respectively.

Table 5 shows a comparison of the geometric mean, median values and 95% confidence intervals of haemoglobin, WBC, lymphocyte subsets and B-2M between HIV-1 seropositive patients and seronegative referents. A significant difference was found in haemoglobin concentration , total WBC, total and percentage lymphocyte, $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, CD4:CD8 lymphocyte ratios and B-2M concentrations but there was no significant difference in $CD3^+$ T-lymphocyte percentage between HIV-1 infected patients and referents.

Table 6 shows the geometric mean and median values of lymphocyte subsets in patients in the four different clinical stages of HIV infection. There was a significant difference in the haemoglobin concentration, $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocyte percentages and CD4:CD8 lymphocyte ratios in all the groups. The proportion of referents and patients in the various clinical stages with $CD4^+$ T-lymphocyte counts at different levels (>400, 200-400 or <200 per ul) is shown in Table 7. One hundred and fourteen (95.8%) of the seronegative referents had $CD4^+$T-lymphocyte values above 400/ul while all patients in clinical stage four had values below this level and 75% of these patients had $CD4^+$ T-lymphocyte counts lower than

200/ul. There was a significant difference in the median B-2M level in all groups ($p< 0.001$). The concentrations of B-2M were significantly correlated to the relative and absolute numbers of CD4 T-lymphocytes and to the CD4:CD8 lymphocyte ratios in patients and in the referents ($p<0.003$).

In the longitudinal study 208 HIV-1 antibody positive individuals were recruited into the study during the period of four years including 155 (74.5%) males and 53 (25.5%) females. The composition of the study population was comparable to that of HIV-1 seronegative referents in the same work places who included 416 individuals of whom 313 (75.2%) were males and 103 (24.8%) were females. The mean age of HIV-1 infected individuals was 32.5 and 28.9 years for males and females respectively and the difference in the mean age between the two sexes was statistically significant ($p= 0.002$). Until the end of the third year of follow up forty three individuals became HIV-1 antibody positive and of these 31 individuals (21 males and 10 females) have been followed intensively with studies of their laboratory parameters.

Table 8 shows the mean counts of WBC and lymphocyte subsets, concentration of B-2M and concentration of soluble CD30 among the seroconverters. In eight of the seroconverters in whom laboratory values for lymphocyte subsets, B-2M and soluble CD30 before seroconversion were available, there was a fall of $CD4^+$ T-lymphocyte counts from a mean of 648 cells/ul to 548 cells/ul in the first year of seroconversion, while the percent $CD4^+$ T-lymphocytes dropped by 36% of the original value during the first year after seroconversion followed by gradual fall thereafter. A similar fall of CD4:CD8 ratios was also evident during the follow up period. The $CD8^+$ T-lymphocyte counts in the cohort doubled in the first year with a gradual rise thereafter.

There was a gradual rise of concentration of B-2M during the three years of follow up. The level of soluble CD30 rose from a mean of 13.5 U/ml before seroconversion to 33.1 U/ml during the first year after seroconversion with almost the same increase in the second year. It was not possible to test for the significance of the findings in the seroconverters due to the small number of individuals studied so far.

Table 5. Geometric mean and median (95% confidence interval) of haematological and immunological parameters determined in blood donors (referents) and in HIV-1 antibody positive individuals.

	REFERENTS n=119[1]		PATIENTS n=183[1]		
	mean	median	mean	median	p.value
Haemoglobin[1]	11.6	12.0 (11.0,12.3)	8.3	8.1 (7.5,9.1)	<0.001
WBC $X10^9/L$	5.7	5.6 (5.4,6.0)	5.1	5.4 (5.1,5.9)	0.0082
Lymph.%	34.4	35.0 (32.9,36.1)	29.6	31.0 (27.6,31.6)	0.0035
Lymph/ul	1963	1890 (1843,2091)	1517	1521 (1385,1661)	<0.001
CD3+T %	70.9	72.0 (69.8,72.1)	69.4	71 (67.7,71.2)	0.8468
CD3+T/ul	1404	1366 (1313,1502)	1053	1120 (955,1160)	<0.001
CD4+T%	40.2	41.0 (38.6,41.8)	13	17 (10.4,14.1)	<0.001
CD4+T/ul	791	780 (735,851)	202	277 (136,205)	<0.001
CD8+T%	27.6	27.0 (26.3,29.0)	46	50.8 (44.6,49.5)	<0.001
CD8+T/ul	542	530 (501,587)	687	684 (597,719)	<0.001
CD4:CD8 ratio	1.45	1.46 (1.35,1.57)	0.34	0.36 (0.21,0.31)	<0.001
B-2M gm/l[2]	1.52	1.5 (1.36,1.70)	3.05	3.05 (2.8,3.3)	<0.001

[1] For Heamoglobin 111 HIV infected individuals were studied.
[2] For B-2M 48 referents and 86 HIV-1 infected individuals were studied.

Table 6. Distribution of medians, means (95 percent confidence interval) of immunological markers in HIV-1 infected individuals at different clinical stages.

Parameters	HIV-1 clinical stages.				
	I n=52 median mean	II n=50 median mean	III n=41 median mean	IV n=40 median mean	p.value
Lymph%	33 30.6 (27.3,34.2)	30.5 30.8 (26.8,35.2)	29.5 28.2 (23.9,33.3)	30 27.8 (13.2,52.6)	0.6089
Lymph/ul	1770 1678 (1468,1918)	1614 1560 (1296,1878)	1359 1416 (1158,1730)	1150 1327 (1026,1715)	0.2041
CD3+T%	71 69.5 (66.7,72.5)	72 71.7 (68.3,74.3)	71.5 69.9 (66.4,73.5)	69.5 66.2 (61.1,71.7)	0.519
CD3+T/ul	1272 1180 (1020,1366)	1159 1109 (919,1338)	963 1001 (814,1231)	859 862 (652,1141)	0.1428
CD4+T%	30.4 26.6 (21.3,30.8)	20.5 16.3 (13.1,20.4)	9.7 8.9 (6.9,11.6)	4.0 4.3 (3.1,5.9)	<0.001
CD4+T/ul	460 381 (289,504)	321 269 (206,350)	139 114 (81,161)	45 46 (28,78)	<0.001
CD8+T%	37.5 36.3 (32.9,40.0)	49.0 46.6 (42.5,51.0)	56.6 55.3 (51.1,59.9)	60.5 56.3 (51.2,62.0)	<0.001
CD8+T/ul	635 594 (506,696)	725 754 (644,882)	681 670 (546,823)	755 610 (473,787)	0.5657
CD4:CD8	0.74 0.71 (0.55,0.90)	0.46 0.35 (0.26,0.46)	0.15 0.16 (0.12,0.22)	0.06 0.08 (0.05,0.106)	<0.001
B2M gm/l[1]	2.1 2.1 (1.8,2.4)	3.2 2.9 (2.5,3.3)	3.7 3.6 (3.1,4.3)	3.9 3.9 (3.3,4.6)	<0.001

[1] 86 HIV-1 infected sera were tested (clinical stages 1=19, II=25, III=19 and IV=23)

Table 7: The proportion of referents and HIV-1 infected individuals with CD4 T-lymphocyte counts at different levels.

CD4+T lymphocyte	Referents	HIV-1 infected individuals			
		Clinical stages			
		1	2	3	4
>400/ul	114/119 (95.8)[1]	37/52 (71.2)	15/50 (30)	5/41 (12.2)	0/23 (0)
200-400/ul	5/119 (4.2)	15/52 (28.8)	25/50 (50)	8/41 (19.5)	10/40 (25)
<200/ul	0/114 (0)	0/52 (0)	10/50 (20)	28/41 (68.3)	30/40 (75)

[1] Percentage within brackets.

Table 8. Trend of the geometric means of haematological and immunological laboratory markers among seroconvertors before and after seroconversion.

	Years after seroconversion.				
	Before	1st	2nd	3rd	4th
	n=8	**n=6**	**n=10**	**n=11**	**n=9**
WBC X 10^9	4.7	6.4	5.2	5.6	6.5
Lymph/ul	1758	2263	2011	1767	2315
Lymph %	37.2	37.4	37.5	37	35.3
$CD3^+$ T %	61.6	63.2	69.1	66.7	73.5
$CD4^+$ T/ul	648	548	499	321	314
$CD4^+$ T %	39.1	24.8	22	17.7	14.3
$CD8^+$ T/ul	389	723	810	633	1105
$CD8^+$ T %	23.4	34.1	39.3	41.9	51.3
CD4:CD8 ratio	1.6	0.7	0.6	0.5	0.3
B-2M gm/l*	1.3	1.5	2.4	2.7	ND**
CD30 I/ml*	14	32.2	45	38.9	ND**

* The number of samples tested were 28, 23, 25, 13 and 3 for before, 1st, 2nd, and 3rd year after seroconversion respectively.

** Not Done

7.0. DISCUSSION:

7.1 Study 1

It is important to establish reference values and study the variation of the different laboratory values in different healthy population groups due to variation which has been reported in relation to age, sex and ethnic groups[138-147]. Few studies have shown differences in the lymphocyte subsets in relation to age and sex[138-140]. In those studies there was a change in the levels of $CD4^+$ and $CD8^+$ T-lymphocyte counts with advancing age[138]. Among teenagers the proportion of $CD4^+$ T-lymphocytes were similar in younger boys and girls with slight increase in older girls; for the $CD8^+$ T-lymphocyte counts, teenage girls had higher values than boys. After the age of 20 years boys had higher $CD8^+$ T counts than girls[138].

In the present study there were significant sex differences in the percent $CD3^+$, $CD4^+$ T-lymphocytes and CD4:CD8 ratios which are in agreement with previous reports[138-140]. Controlling for age differences in sexes did not change the sex differences in percent $CD3^+$, $CD4^+$ T-lymphocyte counts and concentration of B-2M. The reasons for these sex differences can be either due to genetic or hormonal differences. The consequence of these sex differences of $CD4^+$ T-lymphocytes in relation to disease progression in HIV infected individuals need to be studied further. In the present study, percentage lymphocytes were more reproducable than absolute counts an observation which is in agreement with previous reports[92]. Lack of correlation between $CD4^+$ T-lymphocyte counts and percentages in our population could be caused by methodological problems like change in cell counters or in technical staff during the four years of followup. Consequently, it will be difficult to adopt the figures widely proposed by Centres for Disease Control which correlate 20% $CD4^+$ T-lymphocytes to 400 cells/ul of $CD4^+$ T-lymphocytes. However, an on going study in our laboratory

using the same cell counter and personnel, appears to be showing a better correlation between $CD4^+$ T-lymphocyte percentages and counts.

It is important to establish reference values in a population in order to delineate between normal and abnormal findings. Reference values of white cell counts among different racial groups have been shown to vary[141-147]. African women have been found in one study to have significantly lower total WBC and neutrophil counts compared to women from other ethnic groups probably due to genetic constitution [145]. In another study the total WBC counts were similar in different racial groups[147] while no ethnic differences were found in neonatal total WBC counts, neutrophils, lymphocytes, monocytes or eosinophils[146]. Reference values are obtained from a healthy population which is often difficult to define due to presence of sub-clinical infections and individual factors which may have an influence on the parameter in question. The reference values in relation to sex obtained in this study are the first to be described in our population and as previously shown, they don't seem to differ so much when compared with those obtained by other investigators[98,99]. The role of the laboratory in establishing lymphocyte subset reference values depends on the ability to conduct precise laboratory techniques with well established quality assurance system especially when doing flow cytometry[148].

There are a few documented studies addressing the variation of repeated lymphocytes subsets within an individual[103]. In the present study there was more variability of the laboratory parameters in males in comparison with females. However, percent $CD4^+$ and $CD8^+$ T-lymphocytes and CD4:CD8 ratios did not significantly change in both males and females in the follow up period. This finding which is in agreement with previous reports[149,150], emphasizes the reproducibility of percentage lymphocytes as

compared to counts. This is an important finding in that percent $CD4^+$ T-lymphocytes is a potential stable prognostic marker which does not vary over years of follow up in healthy individuals.

T-lymphocyte subset counts determination by flow cytometry are a derivative of total peripheral white blood cell count and the differential lymphocyte determination. Variation in any of these two measurements will affect all consequent counts. In this study a significant difference was observed in the differential lymphocyte counts but not in the total peripheral WBC counts when measured repeatedly for four years. It can be speculated that the significant differences which were found in the $CD4^+$ and $CD8^+$ T-lymphocyte counts could be due to the variation of percentage lymphocyte counts and not due to variation of total peripheral WBC counts. It is difficult to establish the cause of the variation which could be due to technicalities or due to presence of various infections which affect the differential leucocyte count such that a rise of percentage neutrophils will also affect percentage lymphocytes. In the present study the differential leucocyte counts were done by flow cytometry in order to obtain consistent results, thus reducing human error when performing such counts. Flow cytometry is the most common technique used in determining T-lymphocyte subsets. It needs an expensive equipment, well trained manpower and established quality assurance system[45]. For this reason, it has been realised that while simpler methods of determining lymphocyte subsets have shown promising results[111,154], alternative laboratory methods either used separately or together with T-lymphocyte subsets are needed especially for use in less well equipped laboratories.

It is well established that there is a circadian variation of the $CD4^+$ T-lymphocytes which parallel variation of $CD3^+$ T-lymphocyte counts but not of $CD8^+$ T-lymphocyte

counts and plasma cortisol levels[105]. In the present study the confounding effect of circadian rhythm was partly controlled by taking blood specimens during the same time period in a day. Exercise leads to increased levels of WBC, drop in the levels of $CD3^+$ and $CD4^+$ T-lymphocyte counts but no change in the $CD8^+$ T-lymphocyte counts[152]. The present study population comprised of hotel workers with varying levels of physical exercise among them depending on the nature of their work, but this issue was not tackled in the study. In addition smoking and pregnancy states were not controlled for in the present analysis although these factors are known to affect $CD4^+$ T-lymphocyte counts[80] . It would therefore be of interest to find out the extent to which

these factors contributed to variability in the $CD4^+$ T-lymphocyte counts in our population.

7.2. Study 2

The cross-sectional study of T-lymphocyte subsets in HIV infected Tanzanian patients clinically staged according to the proposed WHO system showed that the percentage and absolute number of $CD4^+$ T-lymphocytes declined in each of the four clinical stages representing increasing clinical severity of infection. These results are in general agreement with those obtained from cross-sectional and longitudinal studies in developed countries showing a correlation between a gradual $CD4^+$ T-lymphocyte depletion and disease progression[79-81,97,98,153]. In the present study as well as in previously reported studies, the number of $CD8^+$ T-lymphocytes first increased with increasing clinical severity but then decreased in the most advanced clinical stage[80,81,97,98,153].

The median values of T-lymphocyte subsets in HIV-1 seronegative healthy individuals in Tanzania were similar to the median values reported in seronegative individuals in USA and Europe[99,107]. The World Health Organisation (WHO) has proposed a clinical staging system for HIV infection suitable for use in developing countries since it does not require determination of laboratory markers[137]. The WHO staging system also includes a proposal for subdivision of the clinical stages on the basis of the number of lymphocytes and/or $CD4^+$ T-lymphocytes. The possibilities of determining T-lymphocyte subsets by flow cytometry in developing countries are limited because of the sophisticated technology and high costs involved. Simpler methods for T-lymphocyte subset determinations in developing countries have recently been described[111,150] and are being evaluated in relation to flow cytometry in a multicentre study being coordinated by the WHO[154].

The median time from HIV infection to AIDS in developed countries is estimated to be approximately ten years[40], however the incubation period can be as short as few months[47] and some HIV-1 infected individuals remain asymptomatic for more than 13 years[89]. This highly variable rate of progression reflects complex interactions between multiple variables of the HIV and host immune responses. Study of the natural history of HIV infection may provide insight into these differences in the HIV pathogenesis which can then be used to formulate appropriate strategies for therapy and prevention.

In addition to $CD4^+$ T-lymphocyte counts, serum concentrations of B-2M correlated well with the clinical stages of HIV infection, showing an increase with advancing clinical disease as previously demonstrated in other similar studies[90,91,95]. In a previous study in Tanzania, very high levels of B-2M (>5mg/ml) in AIDS patients were found to be associated with a high risk for death within a few weeks[121]. Among

the seroconverters percent total T-lymphocyte counts showed a gradual rise from a value of 64 % to 73.5 % while $CD8^+$ T-lymphocyte percentages increased from 31.4% to 51.3%. Such results are in agreement with reports of other studies done elsewhere[90,91] and shows the importance of having a uniform cohort when studying the natural history of a disease. The absolute and percent $CD4^+$ T-lymphocyte counts before seroconversion were within the normal reference range of our population as seen in the crossectional study. The rate of fall of absolute $CD4^+$ T-lymphocyte counts of about 100 cell/ul per year compares well with previously reported findings[92]. The fall of CD4:CD8 lymphocyte ratio also agrees very much with results obtained from percentage $CD4^+$ and $CD8^+$ T-lymphocyte counts from which it is derived. In this group the level of B-2M showed a gradual rise one year after seroconversion. It has been well established that B-2M is a good marker in predicting death in HIV-1 infected patients when serum levels are above 4.0 mg/ml[121]. On the other hand the change in the level of sCD30 was remarkable, with levels almost doubling one year after seroconversion from 14 U/ml to 32.2 U/ml . This is in agreement with previous findings which established sCD30 levels below 20 U/ml to be normal[135]. These immune activation markers are not specific since they are elevated also in several other clinical conditions[120,133,134].

The natural history of HIV infection among different individuals is sometimes variable with respect to rates of progression to AIDS[1,2]. In this study there were two unusual HIV-1 infected individuals. One was infected before 1991 and has maintained $CD4^+$ T-lymphocyte counts between 580/ul and 870/ul with a CD4:CD8 ratio above 1.0 and the level of B-2M increased from 1.9 gm/dl in 1991 to 2.2 in 1993. The other individual seroconverted at the end of 1991 and after three years of follow up he had a

mean $CD4^+$ T-lymphocyte count of 570 cells/ul and mean CD4:CD8 ratio of 1.0 but the level of B-2M did not change. These two individuals probably represent potential long term HIV survivors[89].

8.0. CONCLUSION.

There appears to be significant sex difference of T-lymphocyte subset counts and B-2M concentration. With the exception of percentage $CD4^+$ and $CD8^+$ T-lymphocytes, counts of lymphocytes subsets and B-2M concentrations vary in the same individuals over a period of time. These factors need to be considered when interpreting and evaluating these parameters in an individual. Haemoglobin concentration, $CD4^+$ T-lymphocytes, CD4:CD8 lymphocyte ratios and serum levels of B-2M correlated strongly with the clinical stages of HIV infection in adult Tanzanian patients. The total WBC counts, total lymphocyte counts and total T-lymphocytes were not significantly different in the four clinical stages of HIV-1 infection. It is concluded that B-2M concentrations can be used as a prognostic marker in HIV infection in developing countries when T-lymphocyte subset determinations cannot be done. Longitudinal studies are needed in developing countries to define better the prognostic value of staging using a combination of clinical and laboratory markers.

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