THE EFFECT OF

ANGIOTENSIN CONVERTING ENZYME INHIBITION

ON

NEPHROPATHY ASSOCIATED WITH

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

BY

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AUTHOR'S DECLARATION

This study represents work done by the author. When use was made of the work of the others, it has been duly acknowledged in the text. The work has not been submitted in any form to any other university.

The work was carried out under the supervision of Professor AGH Assounga in the Department of Medicine, Nelson R Mandela School of Medicine, University of KwaZulu Natal, Durban with the co-supervision of Professor S Naicker, Division of Nephrology, Department of Medicine, University of Witwatersrand, Johannesburg.

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ACRONYMS AND ABBREVIATIONS

ACE, angiotensin converting enzyme

ACEI, angiotensin converting enzyme inhibitor

AIDS, acquired immunodeficiency syndrome

AIH, autoimmune hepatitis

ANF, anti-nuclear factor

ARC, aids related complex

ARF, acute renal failure

ARV, anti-retroviral therapy

ATN, acute tubular necrosis

AT II, angiotensin II

bDNA, branched –chain deoxyribonucleic acid

bFGF, basic fibroblast growth factor

CAPD, continuous ambulatory peritoneal dialysis

CDC, Center for Disease Control

CICs, circulating immune complexes

CMV,Cytomegalo virus

CNS,central nervous system

Cr Cl, creatinine clearance

CRF,circulating recombinant form

CVA, cerebrovascular accident

DARC, Duffy antigen/receptor for chemokines

DNA, deoxyribonucleic acid

ESRD, end stage renal disease

ELISA, enzyme-linked immunosorbent assay

FITC, fluorescine isothiocyanate

GFR, glomerular filtration rate

HAART, highly active anti-retroviral therapy

H&E, haematoxylin and eosin

HIV, human immunodeficiency virus

HIVAN, human immunodeficiency virus-associated nephropathy

HIVICD, human immunodeficiency virus-associated immune complex-mediated renal disease

IFA, immunofluorescence assay

Ig, immunoglobulin

IL, interleukin

IMF, immunofluorescence

ITG, immunotactoid glomerulopathy

LTRs, long terminal repeats

MHC, major histocompatibility complex

PAS, periodic acid schiff

PIN, plasmacytic interstitial nephritis

RIPA, radioimmunoprecipitation assay

RNA, ribonucleic acid

Se Cr, serum creatinine

SIADH, syndrome of inappropriate anti-diuretic hormone

TGF-β, transforming growth factor-β

TNFα, tumor necrosis factor α

TTP/HUS, thrombotic thromocytopenic purpura/haemolytic uremic syndrome

USRDS, United State Renal Data System

WR, Wasserman reaction

ABSTRACT

About 40 million people worldwide have been infected with the human immunodeficiency virus (HIV) (UNAIDS/WHO, 2004). Sub-Saharan Africa remains the most severely affected region in the world with 25.4 million people living with HIV at the end of 2004. In South Africa, the estimate of HIV-1 prevalence from the national population-based survey in adults aged 15-49 years was about 16% [Shisana *et al.*, 2002]. A study conducted by the Medical Research Council (2004) revealed HIV prevalence of 37.5% among pregnant women in KwaZulu-Natal. An estimated 5.3 million people were infected with HIV at the end of 2003 in South Africa. HIV infection affects multiple organs and the kidney is a common target. Black patients have a relative risk of 51.1 for developing end-stage renal disease (ESRD) from AIDS or an AIDS-defining diagnosis compared with white patients [Barisoni, 2003]. HIV-associated nephropathy (HIVAN) is reported as the commonest form of chronic renal disease in HIV infected patients and is the third leading cause of ESRD in African Americans aged 20 to 64 in the United States [D'Agati *et al.*, 1997; Monahan *et al.*, 2001; USRDS, 2001]. The prevalence of renal diseases associated with HIV infection in Africa and South Africa is unknown. There is a lack of surveillance and reporting for renal disease in HIV positive patients. Therefore, in order to assess the pattern of renal disease among the HIV- infected South African patients, this study was performed.

Early detection of HIVAN may be beneficial in evaluating early treatment in an attempt to protect the kidney from further disease progression. This study, therefore, also attempts to diagnose HIVAN at an early stage.

This is a single center, prospective cohort study. Six hundred and fifteen patients attending the HIV clinic and in-patients from King Edward VIII hospital were screened. Inclusion criteria were HIV seropositivity, proteinuria ranging from persistent microalbuminuria to overt proteinuria. Patients with two positive tests for microalbuminuria at least a month apart were selected as having persistent microalbuminuria . Exclusion criteria were diabetes mellitus, uncontrolled hypertension, known causes of chronic kidney disease and serum creatinine >250μmol/l(i.e to select those patients with early nephropathy). A total of 30 patients (29 blacks; 1 mixed race) were recruited and followed up between April 2002 and September 2004. All patients underwent percutaneous renal biopsy. CD4 counts, urea and electrolytes, serum creatinine, creatinine clearance (Cr Cl), 24 hour urinary protein were done at the study entry and 3-6 monthly thereafter. ANF (anti-nuclear factor), WR (Wasserman reaction), Hepatitis B and C were also done. Patients in this study were not on anti-retroviral therapy. None of them had used heroin or any illicit drugs in the past.

Total of 11 patients with proteinuria <1gm/24hours were randomised to receive either Perindopril (6 patients) or no treatment (5 patients, as control). All 19 patients with proteinuria >1gm/24hours were put on Perindopril. HIVAN was found in 25 (86.2%) patients with a mean CD4 count of 232 (3 - 586). Of these 25 patients, 6(24%) had microalbuminuria. Altogether, 7 patients with persistent microalbuminuria were biopsied and 6 (85.7%) showed HIVAN with a mean CD4 count of 216/mm^3 and mean creatinine clearance of 132 ml/min. Other biopsy findings included: membranoproliferative nephropathy in 2 (7%)and interstitial nephritis in 3 (10%). Four patients with HIVAN had associated membranous nephropathy. In patients with proteinuria <1gm/day, ACE inhibition leads to abrogation of proteinuria , whereas it

was non-significantly worsened in controls (p=0.357). Serum creatinine and Cr Cl improved non-significantly with ACE therapy (p=0.3327 and 0.405 respectively), but significantly worsened in the control group (p= 0.047 and 0.042 respectively). Patients with proteinuria >1 gm/day on ACEI therapy had a significant reduction in proteinuria (p=0.003); in addition, renal function was preserved. When the 2 treatment groups were compared, no deterioration of Se Cr, Cr Cl and proteinuria was found in patients with protinuria of <1 gm/day, whereas improvement trends of Se Cr and Cr Cl with significant reduction of proteinuria (p= 0.006) was noticed in the >1 gm/day group. The effect of ACEI therapy in delaying disease progression was assessed by comparing the percentage of patients whose Cr Cl had halved at the end of study. No patients from the group with proteinuria of < 1 gm/24 hr on ACEI therapy had their Cr Cl halved, whereas 1 out of 5 (20%) patients from the control group had Cr Cl halved at the end of the study. From the >1 gm/day group, 2 out of 19 (10.5%) patients showed halving of Cr Cl , implying the renoprotective effect of ACEI with better protection when commenced at the early stage of proteinuria.

In conclusion, HIVAN is the commonest renal biopsy finding amongst our study patients with HIV infection. ACE inhibition was found to be effective in early renal disease associated with HIV infection, presenting with any degree of proteinuria. Microalbuminuria is a manifestation of HIVAN in our study patients. Most patients with HIVAN had low CD4 counts (<250/mm^3). Screening for persistent microalbuminuria may be beneficial in HIV-infected patients with low CD4 count. Renal biopsy may be a consideration in those with persistent microalbuminuria, in order to diagnose HIVAN at an early stage to achieve the maximum benefits of ACE inhibition. Further prospective randomised controlled trials of ACEI and ARV therapy are needed to support our findings.

CHAPTER 1

INTRODUCTION

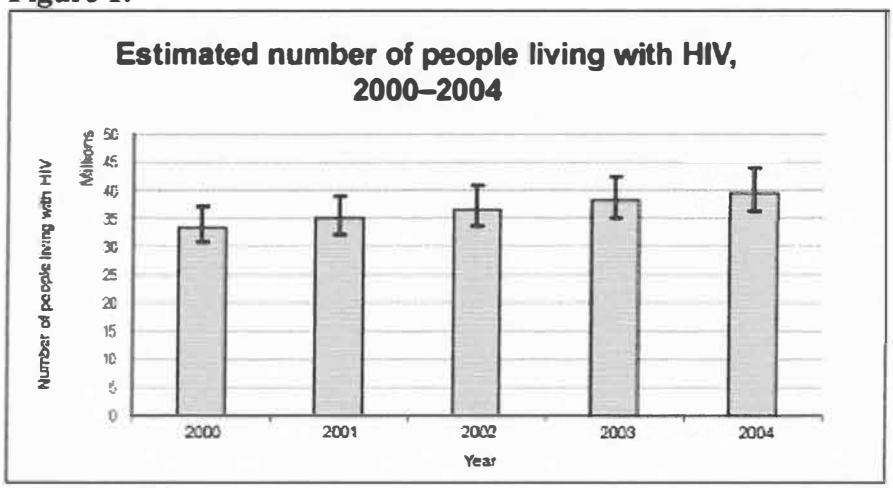
1.1 Global impact of HIV/AIDS

Sixteen thousand persons are estimated to be newly infected with the Human immunodeficiency virus (HIV) each day worldwide; among them, more than 40% are women, more than 50% are 15- to 24-year-olds, and 10% are children age 14 years or younger. About 40 million people worldwide have been infected with HIV (Figure 1). Sub-Saharan Africa remains the worse affected region in the world with 25.4 million people living with HIV at the end of 2004[UNAIDS/WHO,2004] (Figure 2). Just under two thirds (64%) of all people infected with HIV are in sub-Saharan Africa, as are more than three quarters (76%) of all women living with HIV. In this region, HIV seroprevalence rates have been among the highest; for example serologic surveys conducted among pregnant women in Botswana, Uganda, Zambia, Malawi and South Africa have found infection rates of 20% to 35% in urban areas and about 10% in rural areas [US. Bureau of the Census, 1995 & 1998]. HIV seropositivity has been found in 7.4% of the population aged 15 - 49 years [Sande and Volberding, 1999]. In South Africa, an estimated 5.3 million people were infected with HIV end-2003 [UNAIDS, 2004]. The estimate of HIV-1 prevalence from the national population-based survey in adults aged 15-49 years was about 16% [Shisana *et al.*, 2002]. A study conducted by Medical Research Council revealed an increasing prevalence among pregnant women aged 15-24 years, from 23.1% in 2001 to 24.3% in 2003 (Figure 3), with the highest prevalence in KwaZulu-Natal (37.5%) [UNAIDS/WHO, 2004].

HIV is now the number one killer worldwide amongst other infectious agents [Idemyor, 2003]. About 12 million people have died from AIDS over the last 20 years [Thaker et al., 2003]. In South Africa, number of deaths due to HIV related illnesses

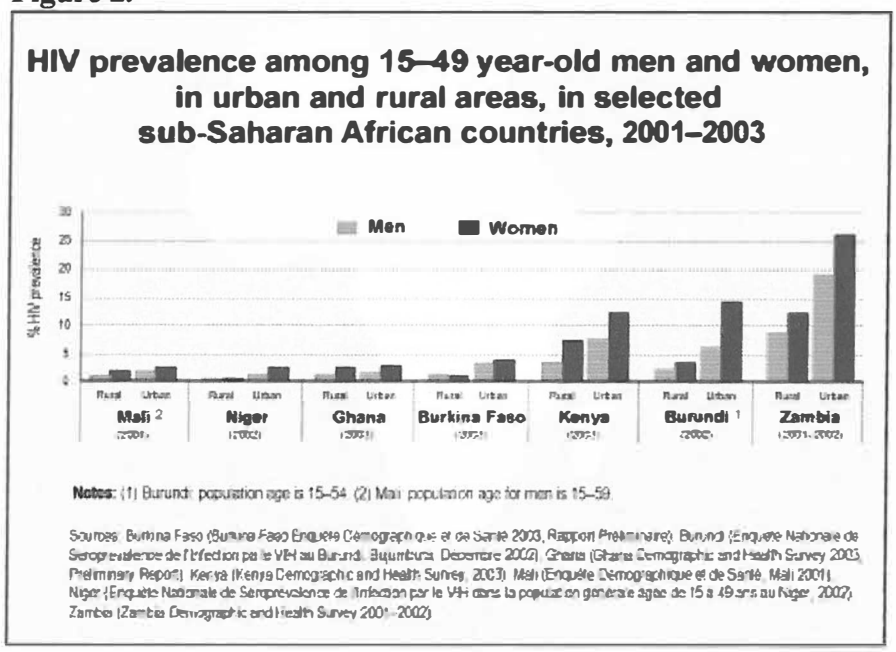
is increasing every year, with nearly half a million people dying in 2003 (Figure 4). The HIV epidemic, therefore, has had a huge global impact, with sub-Saharan Africa being the major target of this deadly disease.

Figure 1.



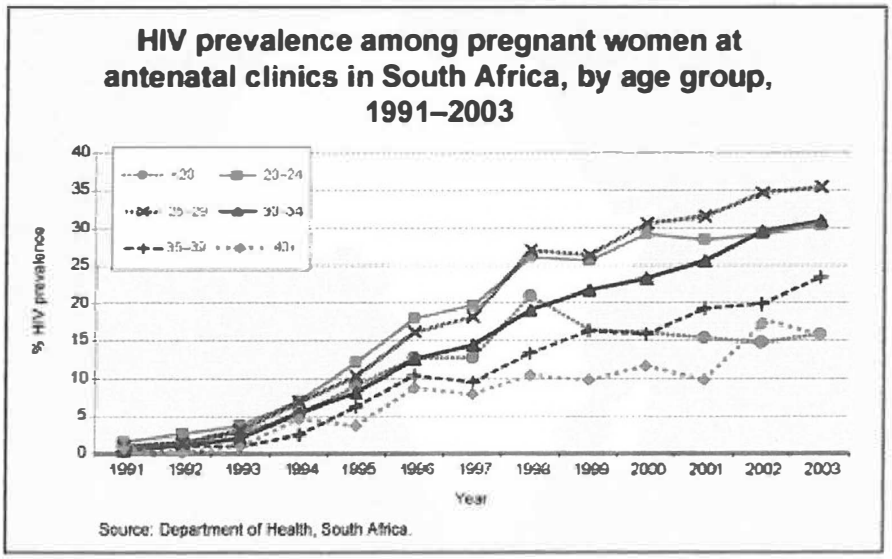
(*UNAIDS/WHO,AIDS epidemic update: December 2004*)

Figure 2.



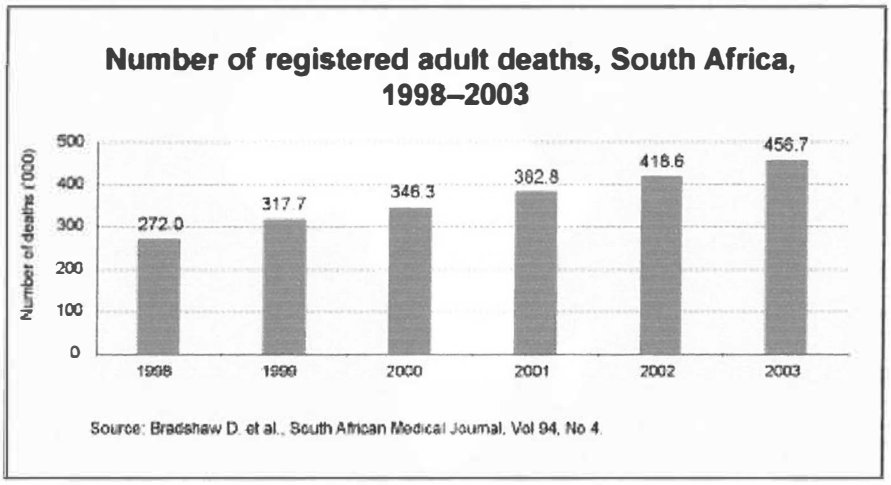
(*UNAIDS/WHO, AIDS epidemic update: December 2004*)

Figure. 3



(*UNAIDS/WHO, AIDS epidemic update: December 2004*)

Figure. 4



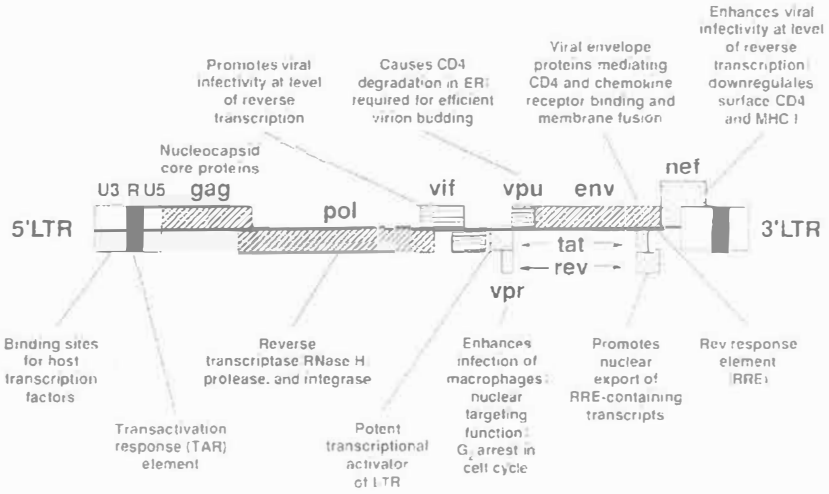
(*UNAIDS/WHO, AIDS epidemic update: December 2004*)

1.2 Characteristics of HIV

HIV-1 is a member of the Lentivirinae subfamily of retroviruses. Other lentiviruses include the simian immunodeficiency virus (SIV), which causes an AIDS-like disease in Asian monkeys; the visna and maedi viruses, which cause severe demyelinating encephalomyelitis and interstitial pneumonia in sheep; the caprine arthritis-encephalitis virus; the equine infectious anaemia virus; and the feline immunodeficiency virus.

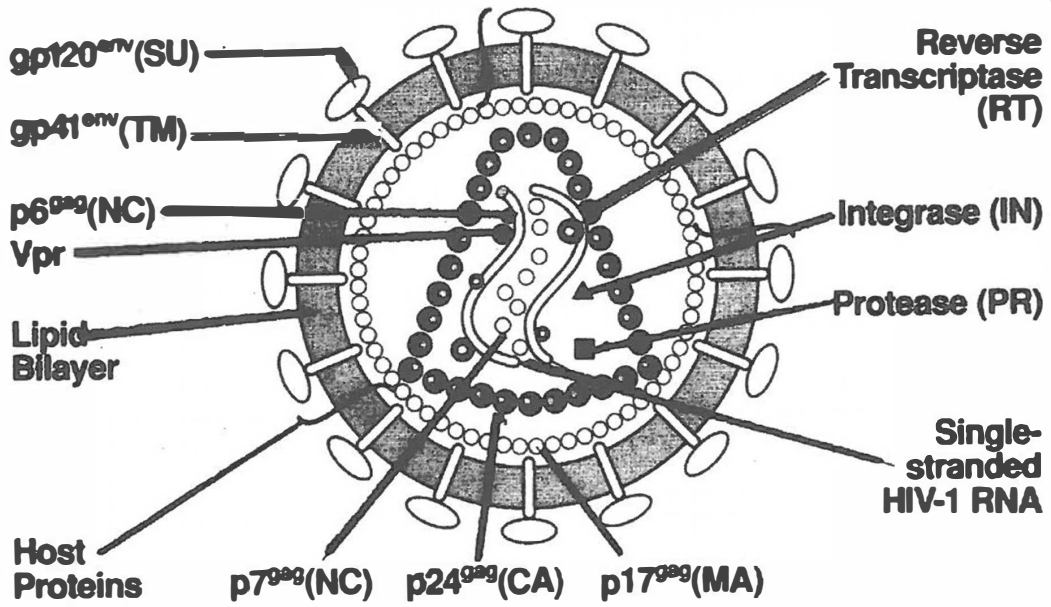
The remarkable feature of the lentiviruses, which differs from other retroviruses, is the complexity of their viral genomes (Figure 5). Most replication-competent retroviruses contain only three genes, *gag, pol,* and *env.* The *gag* and *env* genes encode the inner core polypeptides and envelope proteins of the virus, respectively, whereas the *pol* gene gives rise to the viral reverse transcriptase, integrase, and protease activities. However, HIV-1 contains within its 9-kilobase pair (kb) RNA genome not only these three essential genes but at least six additional genes (*vif, vpu, vpr, tat, rev,* and *nef*). The proviral DNA is flanked by long terminal repeats (LTRs), which are important for both transcriptional regulation and polyadenylation [Sande and Volberding, 1999].

Figure 5. Genomic structure of HIV -1



The HIV1- virion contains an inner conical structure and 72 external spikes formed by the two major viral envelope proteins, gp120 and gp41. The HIV-1 lipid bilayer is also studded with a number of host proteins, including class I and class II major histocompatibilty complex (MHC) antigens, acquired during virion budding. The viral core contains four proteins: the p24 capsid protein, the p17 matrix protein, and the p7 and p6 nucleocapsid proteins, each of which is proteolytically cleaved from a 55-kDa Gag precursor by the HIV-1 protease. The capsid protein forms the chief component of the inner shell of the virion, whereas the myristylated matrix protein is associated with the inner surface of the lipid bilayer and probably stabilizes the exterior and interior components of the virion. The p7 nucleocapsid protein binds directly to the genomic RNA through a zinc-finger structural motif and together with the p6 nucleocapsid, forms the retroviral core. This core also contains two copies of the single-stranded HIV-1 genomic RNA that are associated with the various preformed viral enzymes, including reverse transcriptase, integrase, and protease (Figure 6).

Figure 6. Schema of the HIV-1 virion.



1.3 HIV-1 subtypes

HIV has been divided into two types;

- HIV Type 1 (HIV-1), which is responsible for the global pandemic; and
- HIV Type 2 (HIV-2), which is less pathogenic than HIV-1, and largely restricted to West Africa, with limited spread to other countries [Wilson *et al.*, 2004].

HIV-1 is a highly variable virus. By using nucleotide sequence comparisons, it is further classified into three groups:

- *Group M (major) viruses*: This group is further divided into 10 phylogenically related genetic subtypes or clades (A,B,C,D,F1,F2,G,H,J and K). These subtypes, together with a growing number of circulating intersubtype

recombinant forms, comprise the majority of HIV-1 variants in the world. Subtypes are unevenly distributed around the world with subtypes A,C and D being the commonest in Africa and subtype B dominating North America and Western Europe. Subtype C, in fact, is emerging as most prevalent, being common in India, and the southern African countries of Botswana, Zimbabwe, Malawi, Mozambique, and South Africa, where it is estimated to be responsible for over 90% of infections. Recombinant viruses, which are viruses with mosaic genomes made up of different subtypes, are becoming more common in regions where multiple subtypes are circulating. Numerous circulating recombinant forms (CRF) have been identified, e.g. CRF01_AE, which is a mixture of subtype A and E; and CRF02_AG, which is a mixture of subtype A and G.

- *Group O (outlier) viruses*: These are largely restricted to the central African region.
- *Group N (non-*M; *non-O) viruses:* These are rare and have been identified in only a few individuals in Cameroon.

HIV-1 virus is a rapidly evolving virus due to the error-prone nature of reverse transcriptase and the high viral turnover. The ability of the virus to adapt rapidly and to diversify has the following serious implications:

- It enables the virus to escape detection by the immune system.
- It enables rapid development of drug resistance.
- It may affect vaccine efficacy.
- It may affect accurate diagnosis, especially pertaining to assays based on detection of the virus, such as a viral load assay.

1.4 Laboratory Testing for HIV-1

Since its discovery in 1984, the presence of HIV-1 infection is tested by direct or indirect methods, detecting virus itself or viral antibodies respectively [Grant *et al.*, 1999; Sandler *et al.*, 1988]

1.4.1 HIV Antibody Tests

There are two tests available to detect viral antibodies.

1.4.1.1 Standard Enzyme-linked immunosorbent assay (ELISA)

This test has been used widely from 1985, based on the technology to perform ELISA available in earlier years. Patient's serum is added to a microwell plate coated with antigens derived from an HIV-1 tissue culture lysate. Bound anti-HIV antibody from the infected patient is detected via a goat anti-human antibody labeled with an enzyme designed to react with a specific substrate. The cleaved substrate product yields a colour that can be measured photometrically; but this test may yield false positive results due to the presence of cross-reacting antibodies, such as those against class II human leukocyte antigens (HLA-DR-4 and DQw-3). These antibodies are frequently present in multiparous women and persons who had received blood transfusions. Other autoantibodies such as parietal cells or smooth muscle antibodies, antinuclear antibodies, antimitochodrial antibodies and anti-T-cell antibodies also can lead to false-positive results. False-negative results can also occur during the seronegative window period of 1 to 3 months (i.e between the time of initial infection and the development of a detectable immune response). Other causes of false-negative reactions include replacement transfusions, bone marrow transplantation, and

commercially available test kits that detect antibody to p24 only (e.g the ELISA test using recombinant p24 antigen). However, a positive ELISA test result should be classified as " HIV reactive" *not positive,* and should be confirmed by a supplemental test such as Western blot.

1.4.1.2 Western Blot (WB)

This is the most commonly used supplemental test. It is performed by separating tissue culture-derived HIV-1 proteins and glycoproteins via polyacrylamide gel electrophoresis, transferring (blotting) the separated proteins onto nitrocellulose paper, incubating the cut strips of nitrocellulose paper with patient serum, and detecting anti-HIV antibodies that have bound to the HIV-associated proteins at the precise point at which they migrated in the gel. Through this procedure, the antibody reactivity against specific antigens can be determined. Antibodies can be identified to six major HIV proteins: gp 41 and gp 120 (envelope), p24 and p17 (core), and p66 and p31 (reverse transcriptase). Western blot (WB) test result, however, can be non-specific and indeterminate. Therefore, several organizations such as the World Health Organization (WHO) and the Centres for Disease Control and Prevention (CDC) have proposed criteria for interpreting Western blot reactivity. The two most commonly used criteria recommend that reactivity to (1) p24 *and* gp41 or gp120/160 or (2) p24 or p31 *and* gp41 or gp120/160 be observed to diagnose HIV-1 positivity

Approximately 15 to 30% of ELISA-negative samples produce indeterminate results on WB. Among ELISA-reactive samples, 4 to 20% can show indeterminate WB results [Kleinman *et al.*, 1990; Genesca *et al.*, 1989].

1.4.1.3 Radioimmunoprecipitation Assay (RIPA)

This test requires ongoing HIV-1 replication in lymphocytic cell lines which occurs in the presence of radiolabelled amino acids which are then incorporated into viral proteins. A cell lysate is then prepared and is incubated with patient serum. Anti-HIV-1 antibodies present in the serum react with the radiolabelled antigens and form immune complexes, which are removed by incubating the reaction mixture with protein A-coated beads. These beads bind the Fc portion of immunoglobin molecules and are separated from the reaction mixture through centrifugation. By adding a detergent, antibody-antigen complexes are eluted from the separated beads. The immunoprecipitants are then run through an electrophoretic gel, which separates them according to their molecular weight as in the Western blot method. An audioradiograph of the gel provides a banding pattern which is very similar to that of the Western blot test.

This assay is considered more sensitive and specific than the Western blot test. However, it is more expensive, time consuming and labour-intensive, because of which it is usually reserved for difficult-to-diagnose cases.

1.4.1.4 Indirect Immunofluorescence Assay (IFA)

The indirect immunofluorescence assay (IFA) requires preparation of HIV-1 antigens that are expressed on infected cells, similar to RIPA. Infected cells are placed on glass slides in a fixed monolayer and are then incubated with patient serum which contains anti-HIV-1 antibodies. These antibodies bind to antigens which are expressed on the surface of infected cells. Bound antibodies are then detected with anti-human

antibody that has been labelled with fluorescein isothiocyanate (FITC). After further necessary processing, the slide is examined under a fluorescent microscope, and the number of cells, the intensity and pattern of staining are assessed. IFA can detect the earliest serologic response against the virus, which is the formation of immunoglobulin M (IgM) antibodies, during acute infection. However, due to time, expense, and expertise required for this test, it is impractical to be used routinely.

1.4.1.5 Other Anti-HIV-1 Antibody Tests

Double antigen-capture ELISA tests: These are the third generation of standard anti-HIV ELISA with increased sensitivity which are based on using labelled HIV-1 antigen to detect binding of patients antibodies to beads also covered by HIV-1 antigens. Therefore, these assays detect all classes of antibodies including IgM that form during acute infection. These are more sensitive assays allowing earlier detection of HIV-1 seroconversion.

Detuned ELISA: This is the assay that is intentionally modified to become less sensitive. It involves modification of the sample dilution and sample incubation time. This assay allows detection of recent HIV-1 seroconversion from a single serum sample.

Antibody test using recombinant purified HIV-1 antigens: These recombinant antigens replace viral lysates which contain substantial amounts of host cell antigens that may cross-react with the patient's serum and yield false-positive results. Recombinant virus proteins are more pure, therefore, other serum reactions with host cell are eliminated, and provide increased specificity.

Rapid antibody test: These tests are done by using filter paper embedded with HIV-1 antigens. The serum to be tested is passed through the filter paper. After a wash, the secondary antibodies are passed over the filter, allowing binding to anti-HIV antibodies that were retained. These rapid tests are easier to perform and are designed to be done one at a time eliminating the need for batch testing. However, these test results need to be confirmed by using confirmatory tests such as p24 antigen assays.

1.4.2 Viral Culture Techniques

There are various culture techniques to isolate the HIV-1 virus.

1.4.2.1 Peripheral Blood Mononuclear Cell Co-culture for HIV-1 isolation:

Viable peripheral blood mononuclear cells (PBMCs) from HIV-1-infected patients are collected by centrifugation of anticoagulated whole blood, which are then co-cultured with PBMCs from uninfected human donor that have been stimulated previously. Cell growth in tissue culture is supported by a special medium to stimulate expression of CR4 receptors for enhanced viral replication and proliferation of lymphocytes. The cultures are observed for syncytium formation (i.e multinucleated giant cell formation) as evidence of viral infection in vitro, and for the presence of either HIV-1 reverse transcriptase (RT) activity or HIV-1 p24 antigen production in the culture supernatant. When performed properly, this culture technique is positive in 95% to 99% of HIV-1 infected patients.

1.4.2.2 Quantitative Cell Culture: This technique measures the relative amount of viral load within cells. The culture technique is the same as the previous method

however, decreasing amounts of patient's cells are used to co-culture with fixed amount of donor cells. In this way, fewer patient's cells are introduced into the co-culture system, thereby allowing measurement of relative viral burden.

1.4.2.3 Quantitative Plasma Culture: This is the measurement of free infectious virus in the plasma. This is done through quantitative plasma culture techniques. Serial dilutions of plasma are prepared and mixed with PBMCs from uninfected donor. To ensure accuracy, these tests are done in duplicate or quadruplicate.

1.4.2.4 Ultrasensitive Cell Culture: This method provides the optimal culture condition for tissue reservoirs (such as resting memory CD4+ T lymphocytes) for HIV-1. By doing so, it increases the sensitivity of the standard method, thereby allowing HIV-1 to be cultured from nearly 100% of subjects with suppressed HIV-1 RNA. This test, therefore, can be used in patients who are on antiretroviral therapy. However, the clinical utility of these ultrasensitive cultures remains unclear. A wider range of tests needs to be done to get reproducible results.

1.4.3 P24 Antigen Assays

1.4.3.1 HIV p24 Antigen test

This is an ELISA-based test that detects the presence of free HIV-1np24 antigen in the patient's serum. Free antigen is bound (captured) by specific anti-p24 capture antibodies coated onto a microwell. Bound antigen is detected by specially designed rabbit anti-HIV P24 (detection antibodies), which in turn, are detected by goat anti-rabbit antibodies. The goat antibodies have been conjugated with an enzyme that cleaves a specific substrate, yielding a coloured product. By photometrically

measuring the degree of colour in the well, the amount of HIV-1 p24 antigen can be determined quantitatively. This test can identify p24 antigen before HIV antibodies are detectable in more than 90% of subjects [Gallarda *et al.*, 1992]. More recently, the use of monoclonal anti-p24 antibodies has increased the sensitivity of the assay significantly, allowing p24 antigen levels as low as 7 to 10 pg/ml to be detected reliably.

1.4.3.2 Acidified p24 Antigen Procedure

This is a modified p24 antigen test which increases the sensitivity of this test. This modification is based on the concept that in the presence of significant amounts of anti-p24 antibody, p24 antigen forms antigen-antibody complexes thereby preventing detection of free antigen. Through acidification of plasma, these complexes are disrupted, releasing free antigen for detection by the antigen assay. This technique may be useful in observation of patient responses to antiretroviral therapy, although more recent data indicate that regular p24 antigen testing may be more informative.

1.4.4 Quantitative Viral RNA and DNA Assays

These assays are used to measure the viral load and are based on the Polymerase Chain Reaction (PCR), Branched-Chain DNA (bDNA) and Nucleic Acid Sequence-Based Amplication (NASBA) techniques. HIV RNA and DNA can be detected in almost all HIV-infected patients before HIV antibodies become detectable.

All of the currently available viral load assays have false positive rates of up to 5%, although the mechanism underlying false-positive test results differs between assays.

In practice, the specificity of viral load assays for diagnosing HIV-1 infection is typically between 95% and 99%, which is considerably worse than the specificity of p24 antigen assays and antibody assays,

Therefore, HIV-1 antibody assays remain the gold standard for the diagnosis of HIV-1 infection.

1.5 HIV and Renal Disease

HIV infection affects multiple organs and the kidney is a common target.
The association between HIV and renal disease was first reported in 1984 by investigators in New York City and Miami [Rao *et al.*, 1984; Pardo *et al.*, 1984]. They reported a series of HIV-1 seropositive patients who developed a renal syndrome characterised by proteinuria and progressive renal failure.
A variety of renal syndromes can occur during the course of HIV infection. These can be divided into disturbances of fluid-electrolyte and acid-base metabolism, acute renal failure, immune-mediated glomerulopathies and chronic progressive renal failure.

1.5.1 Fluid-electrolyte and acid-base disturbances

Sodium

Hyponatraemia is the commonest electrolyte disturbance in patients with HIV infection. In hospitalized patients, it was observed in 30% to 56% of persons with AIDS or AIDS-related complex (ARC), as compared with 1% incidence in general medical-surgical inpatients[Anderson RJ, 1986]. In a prospective analysis by Tang et

al, hyponatraemic patients were hospitalized for 17 ± 1 days, with a mortality rate of 36.5%, significantly higher than the 9 ± 1 hospital days and 19.7% mortality rate in the normonatraemic group [Tang *et al.*, 1993] There are many causes that lead to low serum sodium which include vomiting, diarrhoea, reduced intake, insensible losses due to fever or respiratory diseases. The other causes are syndrome of inappropriate antidiuretic hormone secretion (SIADH) secondary to pulmonary and central nervous system lesions and adrenal insufficiency. Nephrotoxic drugs such as Amphotericin can induce renal sodium loss.

Hypernatraemia occurs less commonly in HIV-infected patients. In a survey of 100 hospitalized AIDS patients, the prevalence of hypernatraemic dehydration (serum sodium >149) was less than 5% [Rao TKS, 1998]. Causes include volume depletion and acquired nephrogenic diabetes insipidus secondary to drugs such as Amphotericin and Foscarnet.

Potassium

Hypokalaemia can be found in 17% of patients with AIDS [Peter SA, 1991] in the setting of volume depletion such as gastro-intestinal (GI) loss from diarrhoea or in the presence of metabolic alkalosis or drug induced tubular acidosis due to Amphotericin B, Rifampin and Co-trimoxazole. It can also be a complication of Fanconi syndrome associated with the antiretroviral drug, Adefovir.

Hyperkalaemia has been reported in 16 to 21% of hospitalised AIDS patients. Causes are not different from seronegative patients such as renal failure, acidosis and adrenal insufficiency. Drugs commonly used in treating opportunistic infections such as Trimethoprim and Pentamidine are also known to be associated with hyperkalaemia.

Calcium

Deranged serum calcium levels are frequently observed among the HIV positive patients. About 18% and 3% of hospitalised AIDS patients develop hypo- and hyper-calcaemia respectively. Hypocalcaemia occurs due to hypoalbuminaemia and medications such as Foscarnet, Pentamidine and Didanosine. Increased serum calcium levels are seen in patients with disseminated CMV infection and in those with granulomatous disease.

Magnesium

Hypomagnesaemia as a result of renal magnesium wasting can be seen in patients treated with both Pentamidine and Amphotericin.

Uric acid

Hypouricaemia is observed in 22% of patients with ARC and AIDS [Maesaka *et al.*, 1990], compared to 1% of those admitted for other reasons. It is more common in women and intravenous drug abusers. It results from a defective renal handling of uric acid.

Hyperuricaemia can be found in HIV-infected patients either secondary to dehydration or malignancies. It is also commonly seen with ddI therapy because of its metabolism to hypoxanthine and subsequently to uric acid. Tubular urate deposits have been found to induce ARF in AIDS-associated lymphoma [Ogea Garcia *et al.*, 1989].

Acid-base disturbances

Acute or chronic gastroenteritis occurs commonly in HIV-infected patients, resulting in bicarbonate losses in the stool causing non-anion gap metabolic acidosis. Lactic acidosis can be seen in the setting of sepsis or severe hypoxaemia due to overwhelming respiratory tract infection. Lactic acidosis, not associated with hypoxia or hypoperfusion, known as type B lactic acidosis has been reported in some patients with AIDS.

Respiratory alkalosis can occur due to hyperventilation induced by pulmonary infections. When lungs decompensate or respiratory muscle fatigue occurs late in the course of infection, respiratory acidosis may be a complication.

Fanconi syndrome

Some of the antiviral drugs like Cidofovir and Adenovir have nephrotoxic effects which can cause Fanconi syndrome, manifested by proximal renal tubular acidosis with bicarbonate wasting, hypophosphataemia, glycosuria, aminoaciduria and hypokalaemia. This usually resolves with discontinuation of the drug.

1.5.2 Acute renal failure

Acute renal failure (ARF) is a common complication in patients with AIDS. Valeri *et al* (1991) studied 449 hospitalized AIDS patients and ARF (defined as 2mg/dl [176.8 µmol/l] or greater increase in baseline serum creatinine concentration) was found in 88 (20%) patients . Other reports indicated that the incidence of ARF was 6% to 20% in hospitalized AIDS patients [Rao TKS, 1998].

The aetiology of acute renal failure in AIDS patients is not different from those found in patients without AIDS. The causes can be categorized into pre-renal, intrinsic renal and post-renal [Rao TKS, 1998]). Table 1 illustrates the various causes of ARF in HIV-infected patients. ARF is attributed to ischaemic renal injury secondary to sepsis in about 50%, to nephrotoxic agents such as aminoglycosides, amphotericin, and intravenous radiocontrast agents in about 25%, and to other causes including acute interstitial nephritis, rhabdomyolysis, massive gastrointestinal bleeding, hepatic and cardiac failure in the remaining 25% [Monahan *et al.*, 2001].

Table 1 . Acute Renal Failure in HIV disease

Pre-renal

- Hypovolaemia (diarrhoea, vomiting, CNS infections/tumors)
- **Hypotension** (sepsis, bleeding, fluid loss)
- Hypoalbuminaemia (massive proteinuria, cachexia, third space fluid loss)

Renal

- Acute tubular (hypovolaemia, shock, sepsis, anoxia, and nephrotoxin)
- Rhabdomyolysis (pentamidine, zidovudine, HIV, cocaine)
- Acute azotaemia from nonsteroidal anti-inflammatory drugs
- Allergic interstitial nephritis (trimethoprim-sulfamethoxazole, phenytoin, rifampin, others)
- Plasmacytic interstitial nephritis
- ARF from haemolytic uraemic syndrome
- ARF from thrombotic thrombocytopaenic purpura
- Postinfectious immune complex glomerulonephritis, other glomerulopathies
- Renal oedema from massive proteinuria and severe hypoalbuminaemia
- Multiple myeloma

Post-renal

- Intrarenal tubular obstruction (crystal
- Extrinsic ureteral compression (lymph nodes, tumours) induced from sulfadiazine, acyclovir, protease inhibitors, and tumour lysis syndrome)
- Retroperitoneal fibrosis
- Intrinsic ureteral obstruction (fungal balls, blood clots)
- Bladder and urethral obstruction

[Rao TKS, 1998]

Pre-renal

Pre-renal azotaemia is most often secondary to volume depletion from gastrointestinal losses due to diarrhoea and vomiting, dehydration from poor intake due to CNS involvement, and hypotension resulting from septicaemia. Occasionally, intravascular volume depletion can occur due to sequestration of fluid into third space in patients with hypoalbuminaemia as a result of massive proteinuria, chronic diarrhoea or severe malnutrition.

Renal

Among the intrinsic renal causes, acute tubular necrosis (ATN) is the most common form of acute renal failure. In autopsy series, the prevalence of ATN in AIDS varied between 8% and 30%, but the actual percentage may be higher because renal biopsy was rarely performed in patients with azotaemia [Rao TKS, 1998].However, in recent years, the incidence of severe ATN is decreasing [Rao TKS *et al.*, 1995], due to the overall improvement in the management of AIDS patients. Both oliguric and non-oliguric forms of ATN are common in AIDS patients. The clinical course is variable, ranging from a mild, short duration of oliguria and azotaemia which is self-limited to that of life-threatening uraemia, requiring intensive care management with dialysis and other life support.

Acute interstitial nephritis is also seen in the HIV-infected patients [Monahan *et al.*, 2001]. It may be due to infection or due to hypersensitivity drug reaction from various medications. Infectious agents include candida, tuberculosis, cytomegalovirus and histoplasmosis. Medications frequently used in treating HIV-infected patients with associated diseases and disorders are also responsible for acute interstitial nephritis,

which include penicillin, cephalosporins, ciprofloxacin, trimethoprim sulphamethoxazole, phenytoin, rifampicin and nonsteroidal anti-inflammatory drugs. A rare form of ARF in HIV-infected patients, which is a treatable condition is plasmacytic interstitial nephritis (PIN). Patients present with either mild to nephrotic range proteinuria and azotaemia. Diagnosis is confirmed by renal biopsy which shows normal glomeruli with plasma cell infiltration in the interstitium. It responds well to corticosteroid therapy [Hom *et al.*, 1989].

Post-renal

Post-renal causes of ARF are also common in HIV-infected patients. Crystal deposition in the kidney tubules causing obstructive nephropathy has been seen in seropositive patients receiving medications such as sulfadiazine, intravenous acyclovir, indinavir and foscarnet [Monahan *et al.*, 2001]. Asymptomatic crystalluria can occur in up to 20% of patients who are on Indinavir therapy. Approximately 8% show symptoms of dysuria and renal colic with mild renal insufficiency. Most patients improve with adequate fluid replacement and discontinuation of the drug. Renal tubular obstruction due to urate deposition has been described in patients with AIDS-associated lymphoma who were treated with chemotherapy, resulting in hyperuricosuria from tumour lysis syndrome [Ogea Garcia *et al.*, 1989].

In summary, ARF syndromes occur commonly in HIV-infected patients in protean ways. It is important to look for the causes which are potentially treatable. With early diagnosis and necessary measures, including dialysis and other life supports offered appropriately, can improve the outcome of the patients.

1.5.3 HIV-associated immune complex renal disease (HIVICD)

There are three distinct HIV-associated glomerulonephritides:

- Proliferative glomerulonephritis
- Mixed sclerotic-immune complex nephropathy
- IgA nephropathy [Kimmel *et al.*, 1995; Nochy *et al.*, 1993]

HIVICD occurs much less frequently than HIVAN, although some biopsy series from the United States done in patients presenting with renal impairment, acute renal failure or nephritic syndrome have demonstrated proportions as high as 25% to 33% [Glassock *et al.*, 1990; Kimmel *et al.*, 1993, 1994, 1995; Nochy *et al.*, 1993; Guerra *et al.*, 1987; D'Agati *et al.*, 1997]. European and Asian case series of HIV-infected patients with renal disease who underwent renal biopsies or autopsies show that patients of European descent typically have glomerulonephritis, however, patients of African descent in the same center usually have HIVAN [Hailemariam *et al.*, 2001; Nochy *et al.*, 1993, Casanova *et al.*, 1995; Connolly *et al.*, 1995; Praditpornsilpa *et al.*, 1999; Williams *et al.*, 1998; Kimmel *et al.*, 1995]. This difference in the pattern of renal diseases among the different racial groups suggest a critical role of host or genetic factors in the phenotypic presentation of renal disease in HIV-infected patients.

Disease such as membranoproliferative glomerulonephritis, membranous nephropathy, and postinfectious glomerulonephritis are often seen in the presence of hepatitis B and/or hepatitis C viral infection. These infections are found to be common in HIV-infected patients with renal disease, especially among intravenous

drug users. However, the underlying pathogenesis remains unclear [Weiner *et al.*, 2003].

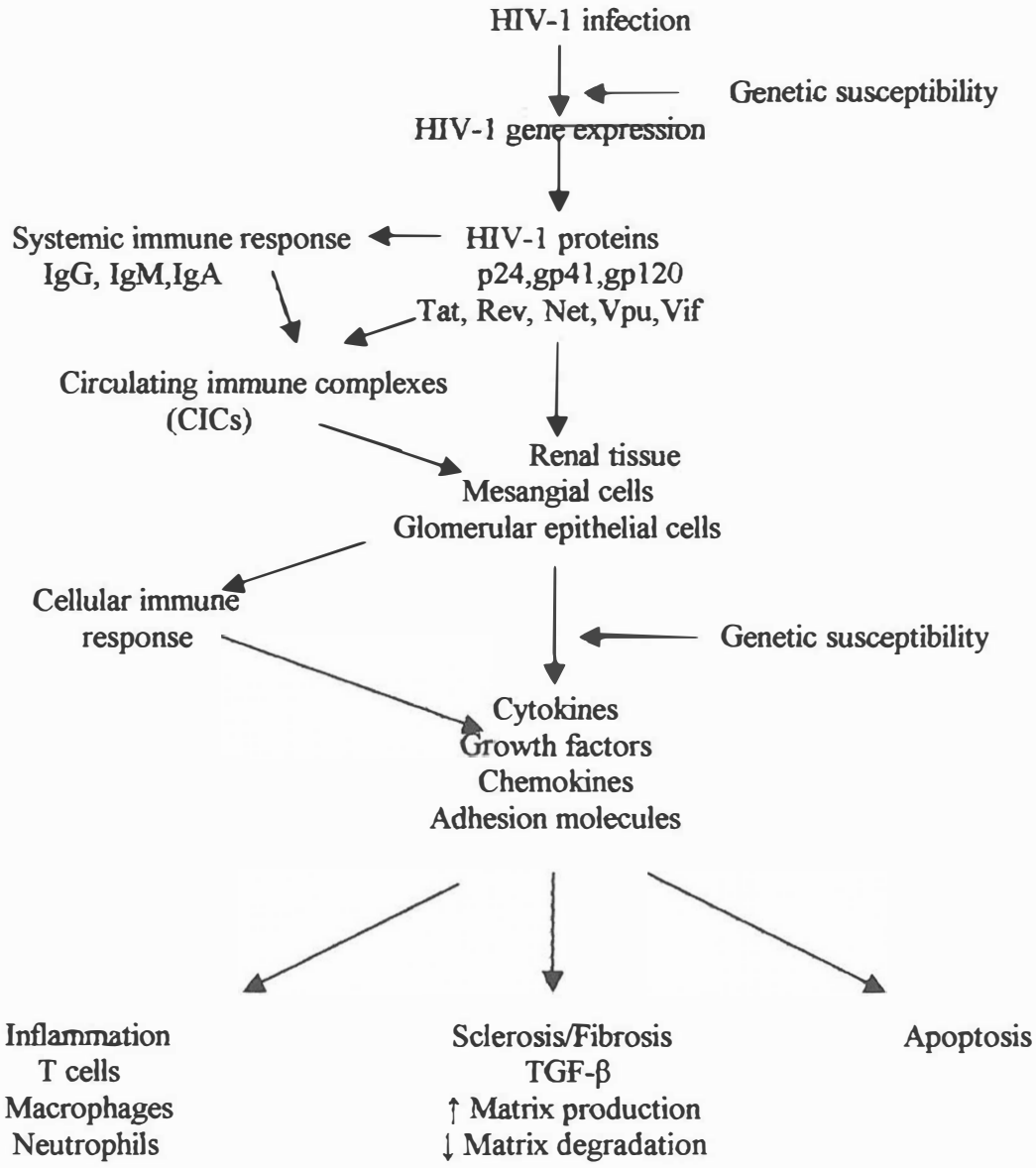
IgA nephropathy also occurs in HIV-infected pateints [Kimmel *et al.*, 1992, 1995, 2000; Katz *et* al., 1992], however, its incidence and prevalence are unknown. In an autopsy study, diffuse mesangial deposits were found in 7.75% of AIDS patients [Beaufils *et al.*, 1995]. IgA nephropathy in association with HIV infection seems to be relatively common in patients of European descent, but uncommon in those of African descent [Weiner *et al.*, 2003; Kimmel *et al.*, 1995; Beaufils *et al.*, 1995], as in HIV uninfected populations [Donadio *et al.*, 2002].

Kimmel et al proposed two potential pathophysiologic mechanisms of HIVICD: (*1*) deposition of immune complexes acting as the inciting factor; or (*2*) in situ immune complex formation initiated by the presence of the HIV genome in renal tissue [Kimmel *et al.*, 1993]. Figure 7 depicts the possible pathophysiologic mechanisms. Primary HIV-1 infection in patients who are genetically susceptible leads to synthesis of HIV-1 proteins. HIV-1 directly infects renal cells and HIV proteins and peptides may stimulate local cellular immune responses. Alternatively, circulating immunoglobulins (IgM,IgG and IgA) may react with HIV-1 proteins resulting in formation of circulating immune complexes, which deposit in renal tissue. A cellular immune response, potentially influenced by host genetic factors, may lead to production of cytokines, growth factors, chemokines, and adhesion molecules. Such a response may lead to various combinations of inflammatory, sclerotic/fibrotic, and apoptotic responses. [Weiner *et al.*, 2003]

The clinical presentation of HIVICD may be quite different from that of HIVAN. Patients with IgA nephropathy present with proteinuria and haematuria, however,

often not with nephrotic range proteinuria [Kimmel *et al.*, 1993]. They tend to have a much more indolent course than those with HIVAN and are less likely to progress to ESRD [Weiner *et al.*, 2003].

Fig. 7. Human immunodeficiency virus-associated immune complex-mediated renal disease (HIVICD)



[Weiner *et al.*, 2003]

1.5.4 HIV-associated thrombotic microangiopathy (HIV-TTP/HUS)

There has been an increasing number of reports in the literature of TTP/HUS in HIV-infected patients over the last few years [Sutor *et al.*, 1999; De Man et al., 1997; Jokela *et al.*, 1987; Ucar *et al.*, 1994; Badesha *et al.*, 1996; Hymes *et al.*, 1997; Berns *et al.*, 1995; Alpers *et al.*, 2003]. TTP/HUS is characterized by microangiopathic haemolytic anaemia and renal insufficiency together with other features such as thrombocytopaenia, fever, and neurological changes. The prevalence of HIV infection among patients with TTP/HUS varies greatly, ranging up to 36% [Berns *et al.*, 1995]. A recent biopsy based retrospective study from France by Peraldi *et al* (1999) suggested the possibility of a higher prevalence of HIV-TTP/HUS than was previously considered. TTP has less severe renal failure and anaemia, and thrombocytopaenia is most prominent. In contrast, microangiopathic haemolytic anaemia predominates in HUS, and renal insufficiency may be extensive [Berns *et al.*, 1995]. However, it may be difficult to differentiate these two entities. The pathogenesis of HIV-TTP/HUS is unknown. In uninfected patients, various aetiologic factors have been identified such as bacterial infections (especially *Escherichia coli*), drugs such as cyclosporin, mitomycin, and bleomycin; and concomittent illnesses such as malignancies, systemic lupus erythematosus and scleroderma [Berns *et al.*, 1995]. However, the contribution of such factors to the pathogenesis of HIV-TTP/HUS is unclear. HIV peptides such as p24 may play a role in the pathogenesis, perhaps because of their cytopathic effect or as a result of changes following the infection of endothelial cells [Del Acro *et al.*, 1993].

The clinical manifestations of HIV-TTP/HUS is similar to the idiopathic forms of the disease, although the demographics differ [Weiner *et al.*, 2003]. HIV-TTP/HUS

occurs mainly in young men, with an 80% male predominance, and a mean age of onset of 35 years. [Berns *et al.*, 1995]. It more commonly occurs in Caucasians than in African Americans or Hispanics. [Berns *et al.*, 1995]. Renal insufficiency is usually mild to moderate. Twenty four hour urinary protein excretion may be in the nephrotic range [Charasse *et al.*, 1991; Meisenberg *et al.*, 1988]. However, the prognosis for patients with HIV-TTP/HUS is significantly worse than for patients with the idiopathic form of the disease, with mortality rates of 66% to 100% [Rao TKS, 2001].

1.5.5 Immunotactoid glomerulopathy (ITG)

ITG is a recognised pathological diagnosis based on renal biopsy specimens since the first description more than 2 decades ago [Martin *et al.*, 2003]. To date, there are several reports of ITG among the HIV-infected patients, either in association with hepatitis C infection or in non-African Americans [Haas *et al.*, 2000; Markowitz *et al.*, 1998; Belgiojoso *et al.*, 1996] although a case of ITG in a HIV positive but hepatitis C-negative African-American has been reported [Martin *et al.*, 2003].

The pathogenesis of ITG is unknown. Possible theories include circulating immunoglobulins with a propensity for tactoid formation that may be polyclonal in nature [Schwartz *et al.*, 2002]. Another recent theory proposes that tactoid formation is localized to the podocyte [Schwartz *et al.*, 2002]. If infection of the podocyte from the HIV occurs, which has been shown by Winston *et al* (2001), CD2ap, a 80-KD protein that normally bind to nephrin in the slit diaphragm of the podocyte is disturbed. This may be a possible mechanism for tactoid formation in the podocytes.

1.5.6 HIV-associated nephropathy (HIVAN)

1.5.6.1 Definition

HIVAN is morphologically defined by a collapsing form of focal segmental glomerulosclerosis (FSGS), glomerular visceral epithelial cell hypertrophy, and prominent tubulointerstitial inflammation with oedema, fibrosis, and microcystic tubular dilatation [*D'Agati et al, 1989; Cohen et al, 1988*]. Endothelial tubuloreticular inclusions (TRI) were also commonly found in HIVAN biopsy specimens before the advent of highly active antiretroviral therapy (HAART) [*D'Agati et al,* 1989]. However, TRI are less commonly found in the post-HAART era [*D'Agati et al, 1998*].The finding of collapsing FSGS, especially with tubular microcystic dilatation in an HIV-1-seropositive patient is almost diagnostic of HIVAN. However, renal manifestations in HIV infected patients can be due to conditions other than HIVAN and renal biopsy is required to establish a definitive diagnosis, even among the HIV seropositive patients [*D'Agati et al, 1997 & 1998*].

1.5.6.2 Prevalence

HIVAN is reported as the commonest form of chronic renal disease in HIV infected patients and is the third leading cause of end stage renal disease (ESRD) in African Americans aged 20 to 64 in the United States [D'Agati *et al,* 1997; Monahan *et al,* 2001; USRDS, 2001]. The true prevalence of HIVAN worldwide, however, is unclear. Initial studies suggested that about 10% of HIV infected patients develop HIVAN [*Pardo et al, 1984& 1987; Gardenswartz et al, 1984; Rao et al, 1987;*

Bourgoignie et al, 1988; Valeri et al, 1991]. However, it appears that it is influenced by the type of study (whether autopsy or biopsy based) and different demographics of the HIV infected population, such as racial group. The occurrence of HIVAN in autopsy studies of AIDS patients varies from 1 to 12% [*Mazbar et al, 1990; D'Agati et al, 1989; Seney et al, 1990; Lopes et al, 1992; Shahinian et al, 2000*]. Renal biopsy series showed a higher prevalence (42 to 64%) than autopsy findings [*Connolly et al, 1995; Winston et al, 1999; Ahuja et al, 1999*].

Since the introduction of HAART, the death rate due to AIDS has decreased markedly in the United States for all racial groups, including African Americans [*CDC, 2001*]. New cases of ESRD due to HIVAN (reported as AIDS nephropathy by the United States Renal Data System) increased rapidly until 1995 and then suddenly began to decrease slightly in 1996, possibly reflecting the effect of HAART [*USRDS, 2001*].However, the rate of decline in the incidence of ESRD due to AIDS nephropathy has slowed. The number of new cases, in fact, increased in 1999. *Ahuja et al* (1999) screened 557 HIV-1 infected patients and reported the prevalence of HIVAN to be 1.79% of all patients and 3.5% among African Americans; based on which, the authors speculated that the incidence of HIVAN may be decreasing.

In Africa, the incidence and prevalence of renal diseases associated with HIV infection including HIVAN is unknown. There is a lack of surveillance and reporting for renal disease in HIV positive patients. It is also likely that many Africans with AIDS die of opportunistic infections early in the course of disease, before HIVAN becomes clinically obvious, as HIVAN is usually a late manifestation of HIV infection. However, its occurrence in the early stage of infection has been reported [Bourgorgnie *et al.*, 1990; Winston *et al.*, 1996; D'Agati *et al.*, 1997].

1.5.6.3 Racial Predilection of HIVAN

The common occurrence of HIVAN among seropositive black and Hispanic patients has been reported previously [D'Agati *et al,* 1997; Soni *et al*, 1989; Cantor *et al,* 1991; Williams *et al*, 1998]. Based on the data from the United States Renal Data System (USRDS), a recent analysis has shown that HIVAN is more strongly associated with black race than any other cause of renal failure with the exception of sickle cell disease [Abbott *et al*, 2001]. Casanova *et al* reported a series of 26 renal biopsies performed on white Italians, none of whom had HIVAN. A similar result was found among HIV infected Thai patients by Praditpornsilpa *et al* (1999). Winston *et al* (1999) and Ahuja *et al* (1999), however, reported HIVAN in 50% and 64% of HIV positive African Americans respectively in renal biopsy studies. Hailemariam *et al* (2001) from Switzerland studied 239 autopsies done on AIDS patients between 1981 and 1989. Different renal abnormalities were found among Caucasian patients; however, the only case of HIVAN was detected in one of six black African patients included in the study.

About 25% of patients with HIVAN have first-degree or second-degree family members with ESRD, and black patients with HIVAN are 5.4 times more likely to have a first-degree or second-degree relative with ESRD than are black patients without renal disease [Freedman *et al,* 1999].

Some investigators proposed Duffy antigen/receptor for chemokines (DARC) as a candidate gene in the pathogenesis of HIVAN, based on a high prevalence of DARC promoter polymorphisms in black patients; Liu *et al* (1999) have shown increased DARC expression in renal tissues from children with HIVAN and haemolytic uraemic

syndrome. However, conflicting results were reported by other investigators [Woolley *et al*, 2001].

1.5.6.4 Pathogenesis

The pathogenesis and mechanisms involved in the rapid progression of HIVAN are currently unknown. Until recently, how HIV infection affected the kidney resulting in HIVAN was unclear. It may be a direct effect of HIV infection or an indirect renal response to HIV-induced immune dysregulation.

There has been conflicting data regarding the presence of HIV-1 in renal cells in clinical HIVAN specimens. In 1989, Cohen *et al* reported the presence of HIV-1 DNA in renal epithelial cells by *in situ* hybridisation. Other investigators reported the finding of HIV-1 by polymerase chain reaction (PCR) in glomerular cells, tubular cells, and infiltrating leucocytes in HIV-infected patients with nephrotic range proteinuria [Ross *et al*., 2002; Kimmel *et al*.,1993]. However, other groups disputed the presence of HIV-1 in renal parenchymal cells in HIVAN biopsy specimens [Barbiano *et al*., 1990; Eitner *et al*., 2000]. Further studies based on animals and humans shed more light onto the pathogenesis of HIVAN.

Mice transgenic for a replication-defective HIV-1 construct lacking the gag and pol genes, expressed under control of the viral promoter (long terminal repeat or LTR), develop proteinuria, renal failure, and histologic renal disease identical to HIVAN [Dickie *et al*., 1991; Kopp *et al*., 1992]. Bruggeman *et al* [1997] later demonstrated that the HIV-1 transgene is expressed in renal glomerular and tubular epithelial cells and that transgene expression in renal epithelial cells was required for the development of the HIVAN phenotype.

Based on animal models, further attempts were made to determine whether HIV-1 infects renal epithelial cells in HIVAN. Bruggeman *et al* (2000) reported a series of 20 HIV-1-seropositive patients with renal disease who underwent renal biopsies. All but one of the patients were black or Hispanic, and 15 had HIVAN. In 11 of 15 patients with HIVAN, HIV-1 was detectable in renal epithelial cells by RNA *in situ* hybridisation.

The mechanism by which HIV-1 gains entry into the renal epithelial cells is unknown. The receptor for HIV-1, CD4, and the major co-receptors for HIV-1, CCR5 and CXCR4 are not expressed in most normal renal epithelial cells. Some investigators have found CD4 and the major co-receptors in cultured renal epithelial cells [Conaldi *et al.*, 1998]; however, no published studies have definitely demonstrated their expression *in vivo* [Eitner *et al.*, 1998 & 2000].Several other co-receptors for HIV-1 have been recently identified [Clapham *et al.*, 2002], however, whether they are expressed in renal epithelial cells remains to be determined.

Renal biopsy examinations of patients with HIVAN have shown both proliferative and apoptotic changes. Renal tubular epithelial cells undergo rapid cell death and apoptosis *in vitro* after infection with HIV-1[Conaldi *et al.*, 1998] The primary process is uncertain; however, because HIVAN kidneys are enlarged, proliferation is most likely the predominant process [Herman *et al.*, 2003].

Transforming growth factor-β (TGF-β) is a fibrogenic cytokine that regulates human immune function and has been shown to regulate HIV replication [Lazdins *et al.*, 1991]. Increased deposition of matrix proteins and increased levels of TGF-β were found in kidneys with HIVAN compared with normal kidneys and with kidneys with

thin basement membrane and minimal change nephropathies. This was also true when compared with kidneys of HIV-infected patients without HIVAN [Yamamoto *et al.*, 1999].

Angiotensin II (AT II) has been shown to increase the cellular synthesis of TGF-β. HIV proteases may interact with the renin-angiotensin cascade and serum angiotensin converting enzyme (ACE) levels have been found to be elevated in patients with HIV infection [Ouellette *et al.*, 1992].

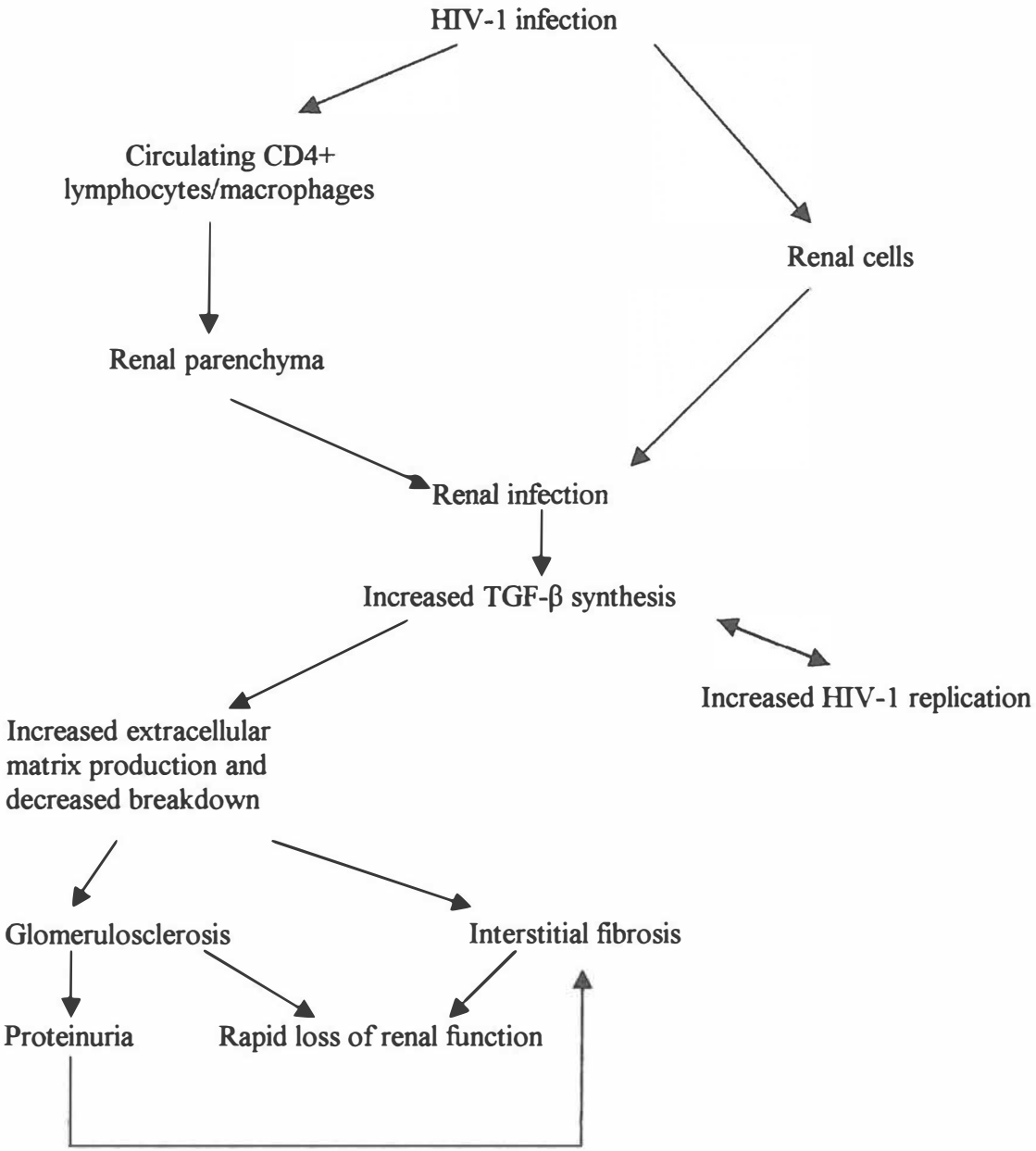
Basic fibroblast growth factor (bFGF) has been linked to renal epithelial cell proliferation in some disease states. In a study comparing HIV transgenic mice with normal mice, transgenic kidneys had increased bFGF and a greater number of bFGF binding sites. Transgenic tubular epithelial cells were found to express bFGF and transgenic epithelial cells or nontransgenic cells treated with bFGF exhibited an increased rate of proliferation compared with controls [Ray *et al.*, 1994].

Infection of renal epithelial cells by HIV-1 has important implications for HIV-1 seropositive patients, not only because it contributes to renal disease but also because the kidney may be an important reservoir for HIV-1[Ross *et* al., 2002]. Bruggeman *et al* (2000) detected HIV-1 in renal epithelial cells by both RNA ***in situ*** hybridisation and DNA ***in situ*** PCR in three patients with undetectable viral loads in peripheral blood. The quasi-species of HIV-1 present in renal epithelial cells clustered separately from sequences derived from the same patients' peripheral blood mononuclear cells. This indicates that HIV-1 infection of tubular epithelial cells represents a viral compartment that is separate from the blood. Therefore, the renal tubular epithelium is a reservoir for actively replicating HIV-1 and may support evolution of viral strains that differ significantly from virus present in a patient's blood. It is unknown whether

the renal epithelium is more likely to serve as a reservoir for drug-resistant HIV-1 strains or whether the renal epithelium is susceptible to currently available antiretroviral drugs.

Host factors appear to play an important role in the pathogenesis of HIVAN. Bruggeman *et al* (2000) studied 7 seropositive individuals without HIVAN and 6 were found to have renal cell infection by the presence of viral mRNA, DNA or both, suggesting that not all patients with renal infection manifest HIVAN. Therefore, host factors must be important in rendering them vulnerable to developing HIVAN. Several studies suggest strong genetic determinants of HIVAN as evidenced by the predominance of the disease in seropositive black patients. Freedman *et al* (1999) reported that patients with renal disease who are infected with HIV were more than five times more likely to have relatives with renal disease than control patients without nephropathy. This data suggest an inherited susceptibility to renal disease among patients with HIVAN. [Kimmel, 2003].

Fig. 8. Schematic depiction of proposed mechanism of HIVAN



[Humphreys MH, 1995]

1.5.6.5 Clinical features

HIVAN occurs almost exclusively in seropositive Black patients, with about 90% of patients being African-Americans. The remaining 10% of cases are observed in mixed heritage or Hispanic patients. This disorder is only very rarely found in seropositive White patients. Patients with HIVAN usually present with varying degrees of proteinuria , azotaemia and hypoalbuminaemia. They are typically normotensive. On renal sonogram, kidneys are echogenic and are normal or enlarged in size. Renal function may progress from relatively normal to end-stage renal disease requiring dialysis over a period of several weeks to months and most patients die within a year [Rao *et al.*, 1987; Carbone *et al.*, 1989; Ortiz *et al.*, 1988]. These characteristics are not generally found in other renal diseases in which glomerulosclerosis is seen, such as diabetic and heroin-associated nephropathy and idiopathic focal segmental glomerulosclerosis.

1.5.6.6 Treatment

HIVAN has become an important cause of renal failure and is the third leading cause of end stage renal disease in adult African-Americans in the United States [USRDS,2001]. However, there have been no prospective randomised controlled studies with any form of therapy to date. Drugs that have been used in the treatment of HIVAN include corticosteroids, angiotensin converting enzyme (ACE) inhibitors and antiretroviral medications.

1.5.6.6.1 Corticosteroids

Several uncontrolled studies were conducted to assess the benefit of corticosteroids in patients with HIVAN. Smith *et al* (1996) performed a prospective observational study in 20 patients with HIVAN who were treated with prednisone and were followed up for a median of 44 weeks. Most patients had advanced renal insufficiency and heavy proteinuria at the time of diagnosis. Seventeen patients experienced an improvement in renal function and reduction of proteinuria. However, several patients had relapses after steroid therapy. At the end of the first year, only two patients did not progress to ESRD. Six patients developed serious opportunistic infections. Altogether, 11 patients died during the study period.

A recent retrospective study evaluated the outcome of steroid therapy in 21 patients with HIVAN and advanced renal failure,13 of whom were treated with prednisone, compared with 8 patients who were not [Eustace *et al.*, 2000]. At 3 months, none of the patients on prednisone had reached ESRD whereas 5 out of 8 patients who were not on prednisone had progressed to ESRD. By the end of 1 year, 8 patients in the treatment group were either dead or on dialysis, compared with 7 patients in the non-treatment group. There was no difference in episodes of serious infections between the two groups after correcting for duration of follow up. However, there was an increased hospital stay in the steroid treated group.

In conclusion, the role and efficacy of corticosteroids in HIVAN remains unclear. Further prospective randomised controlled studies are warranted .

1.5.6.6.2 ACE inhibitors (ACEI)

ACEI have been shown to be effective in reducing proteinuria and progression of renal disease in patients with diabetic and nondiabetic renal disease; therefore, they may produce similar results in patients with HIVAN. In a retrospective case-control study, Kimmel *et al* (1996) reported an increase in renal survival associated with the use of Captopril in 18 patients with biopsy proven HIVAN. Burns *et al* (1997) reported a prospective cohort study of 20 patients with HIVAN and mild renal insufficiency. They were offered Fosinopril therapy however, 8 patients refused the treatment. All were followed up for 12 to 24 weeks. At the end of follow up period, those patients on Fosinopril had significantly lower levels of serum creatinine and proteinuria than those who were not on ACEI treatment. Seven patients in the study were on nucleoside reverse transcriptase inhibitor as a monotherapy. Wei *et al* (2003) performed a prospective study to determine the long-term effect of ACEI on renal survival in HIVAN patients. Forty-four patients with mild renal insufficiency and biopsy-proven HIVAN were included in the study. Twenty-eight patients received Fosinopril and 16 were followed as controls over the study period of 5.1 years. Median renal survival of treated patients was 479.5 days, with only one patient progressing to ESRD. All untreated patients developed ESRD with a significantly reduced median renal survival of 146.5 days. Although there are limitations, these studies suggest a role for ACEI in the management of patients with HIVAN and further prospective studies are needed to determine the optimal role of ACEIs in these patients.

1.5.6.6.3 Highly active anti-retroviral therapy (HAART)

Antiretroviral therapy was first used in a patient with HIVAN in 1989 who showed remission of his nephrotic syndrome for 11 months before relapse [Babut-Gay *et al.*, 1989]. Since then, various investigators have evaluated the use of antiretroviral medication for the treatment of HIVAN. Ifudu *et al* (1997) studied 23 HIV infected patients with proteinuria; 5 of them had biopsy proven HIVAN. All of them were treated with Zidovudine. Fifteen patients were compliant with treatment and none of them had worsening of renal function after a mean follow up of 20.4 months. However, all 8 patients who were not compliant with Zidovudine treatment developed ESRD after a mean follow up period of 8 weeks.

The effectiveness of ARV in HIVAN patients was also documented by other investigators as case reports. Winston *et al* (2001) and Wali *et al* (1998) both described a case report of patient with biopsy proven HIVAN who had renal failure requiring haemodialysis support. They were treated with highly active antiretroviral therapy (HAART), and both of them improved dramatically, resulting in discontinuation of dialysis. Renal biopsies done before and after HAART showed the resolution of the histological features of HIVAN in both cases.

Epidemiological studies also showed the effect of HAART in patients with HIVAN. According to the USRDS data, the incidence of ESRD due to HIVAN has decreased among African-Americans with AIDS since the introduction of HAART in 1996, although less rapidly than the incidence of death due to AIDS. Prospective, randomised studies evaluating the efficacy of HAART in preventing or delaying disease progression are warranted.

1.5.6.6.4 Renal Replacement Therapy

Although rates of HIV-related opportunistic infections and diseases decreased dramatically from 1995 to 1999, coincident with the introduction of HAART (highly active antiretroviral therapy), rates of HIVAN remains stable [Kaplan *et al.*, 2000]. Without any treatment, HIVAN rapidly progresses to ESRD. Patients with ESRD require renal replacement therapy either in the form of haemodialysis, continuous ambulatory peritoneal dialysis (CAPD) or renal transplantation ultimately. A recent US Renal Data System (USRDS) database showed that 6,179 patients with HIV infection had received renal replacement therapy in the United States before May 2000[USRDS, 2001].

1.5.6.6.4.1 Dialysis

The prevalence of HIV infection in various dialysis centres is variable and is dependent on the demographics of the population. In France, a much lower prevalence of 0.36% was reported in a survey of 260 dialysis facilities serving 22,707 patients [Poignet *et al.*, 1999]. In an Italian survey of 27,000 patients, a prevalence of 0.13% was found [Barbiano di Belgiojoso *et al.*, 1998].

During haemodialysis, various cytokines, such as IL-1, IL-6 and TNF-α, are released [Bingel *et al.*, 1988; Herbelin *et al.*, 1990; Varela *et al.*, 2001]. These cytokines have been shown to increase viral replication in vitro [Osborn *et al.*, 1989]. Therefore, HIV-infected patients on haemodialysis appears to be at a theoretical risk of increased morbidity. However, in the HAART era, Ahuja *et al* (2000) did a retrospective study involving 22 HIV-infected patients who were commenced on maintenance haemodialysis and they reported that survival of patients was much improved with the

use of HAART versus less aggressive therapy (one or two antiretroviral medications) . In this study, plasma viral load was also significantly lower in the patients who were on HAART therapy. Ahuja *et al* (2002) also showed an improved survival of HIV-infected patients on dialysis therapy in a later study.

Data on the role of peritoneal dialysis in the treatment of HIV-infected patients with ESRD are limited. There have been suggestions by some investigators that increased protein losses with peritoneal dialysis in these patients may lead to increased morbidity and mortality [Tebben *et al.*, 1993]. Ahuja *et al* (2002) recently compared survival among patients on haemodialysis and peritoneal dialysis in the United States by using the USRDS database and found no difference in survival between two groups, when adjusted for confounding variables.

1.5.6.6.4.2 Transplantation

Another form of renal replacement therapy is renal transplantation. Before the introduction of HAART, an analysis of the USRDS showed that HIV-infected recipients were at increased risk of death and graft loss compared with uninfected recipients of cadaver kidneys [Swanson *et al.*, 2002]. As the survival of HIV-infected patients has improved remarkably in the HAART era, renal transplantation as a form of renal replacement therapy is now being offered to HIV-infected patients with ESRD in some centres. Stock *et al* (2003) studies 14 HIV-infected patients who had kidney transplant (10 patients) and liver transplant (4 patients). Patients were followed-up for means of 480 days and 380 days respectively. Rejection occured in 5 of 10 kidney transplant recipients but did not occur in any of the four liver transplant recipients. There were no evidence of significant HIV progression and no adverse

effect of HIV on allograft function. Abbot *et al* (2004) from the United States recently did a retrospective study on 27,851 kidney transplant recipients with a valid HIV serology and found that 47 patients (0.2%) were HIV infected. All patients in the study were on HAART, had CD4 counts of more than 200, were free from opportunistic infections and had undetectable viral loads. It was found that HIV-infected recipients had improved survival compared with HIV-uninfected recipients, although it was not statistically significant in the adjusted analysis. However, there were limitations in this study. It appears that healthier recipients were selected for transplantation; it was not possible to determine the use of HAART therapy or to determine the clinical stage and other manifestations of disease in HIV-infected patients; none of the HIV-infected recipients had HIVAN and white patients were overrepresented. Although no broad consensus about improved posttransplantation survival for HIVinfected recipients in the modern era should be drawn from this study, it does appear that HIV-infected patients can enjoy successful outcome post-transplantation.

There are other factors that may favour posttransplantation survival as well. HIV-infected recipients may benefit from many serendipitous effects of commonly used immunosuppressive agents. Sirolimus inhibits HIV replication at the transcriptional level [Roy *et al*., 2002] and down-regulates the CCR5 receptor [Heredia *et al*., 2003]. Mycophenolate has inhibitory effects on HIV [Chapius *et al*., 2000],works synergistically with many antiretroviral agents [Margolis *et al*., 2002; Hossain *et al*., 2002] and has been used to treat resistant strains of HIV [Press *et al*., 2002; Coull *et al*., 2001]. Certain calcineurin inhibitors such as cyclosporine has anti-HIV activity [Billich *et al*., 1995; Franke *et al*., 1996]. However, HAART therapy interacts with most immunosuppressive therapy, therefore careful monitoring and dose adjustment is

crucial. In conclusion, the analysis of USRDS transplant population indicates that kidney transplantation in HIV-infected patients is plausible and ongoing. However, the proper use of immunosuppression and HAART therapy still awaits the results of properly designed prospective clinical trials.

CHAPTER 2

HYPOTHESIS AND OBJECTIVES

HYPOTHESIS AND OBJECTIVES

As discussed in the earlier chapter, the impact of HIV infection in South Africa ranges from 16 to 20%, according to population-based and antenatal clinic surveys. This is equivalent to about 8.8 million infected people in South Africa. The true prevalence of renal diseases in the HIV infected population in Africa and South Africa is unknown as there is a paucity of data in this regard. If we consider the commonest form of renal involvement which is HIVAN, at least 10% of the infected population will have this disease. Therefore, about 880,000 South African will present with HIVAN during the course of HIV infection. The actual disease rate which has been mentioned in the initial studies, is likely to be higher than 10%. If we take other renal involvement such as acute renal failure into consideration, the impact on renal services will be enormous. So it is important to look for the prevalence and pattern of renal involvement among the HIV-infected South African population.

In any disease, it is desirable to get the diagnosis as early as possible. Early detection of the disease may be beneficial in evaluating early treatment which may help prevent the progression of the disease. This study, therefore, attempts to detect renal disease at an early stage among the HIV positive patients, in particular, HIVAN.

HIVAN is a disease which almost exclusively affects seropositive black patients. Without any treatment, renal function may progress from relatively normal to end-stage renal disease requiring dialysis over a period of several weeks to months. Although there is no proven effective therapy currently available for such patients, ACE inhibitors have been depicted as an effective form of therapy in patients with

various chronic renal diseases including *idiopathic* focal glomerulosclerosis which shares the same histopathological changes as HIVAN. Therefore, ACE inhibition might be beneficial in such patients.

The other objective of this study is to determine the efficacy of ACE inhibition in preventing renal disease progression in HIV-infected patients from an early stage of renal involvement. At the current time, due to the limited resources, HIV-infected patients are not considered for renal replacement therapy should they develop ESRD. Therefore, if ACEI can be proved to be beneficial in the early stage of HIV associated renal disease, these patients will benefit from such therapy which is financially feasible and cost-effective.

CHAPTER 3

METHODOLOGY

3.1 Ethical approval and patient consent

Ethical approval was obtained from the University of KwaZulu-Natal Ethics Committee (Ref. H109/01). The informed consent literature was available in both English and Zulu versions. Patients also received explanations regarding the following before the consent was obtained:

- aims and objectives of the study
- study drug (Coversyl, an ACE inhibitor) and its possible side-effects
- blood and urine sample collections and
- kidney biopsy

3.2 Patient profile

This is a single centre, prospective cohort study. Patients attending the HIV clinic at King Edward VIII hospital, referrals from Medical Out-Patient Departments and hospital in-patients were screened between April 2002 and April 2004. All the relevant demographic and clinical data were collected.

3.2.1 Inclusion criteria

Inclusion criteria were:

- HIV seropositivity

  (detection of HIV antibodies by ELISA test and confirmed by Western Blot)
- Proteinuria ranging from microalbuminuria to overt/ macro-proteinuria

3.2.2 Exclusion criteria

Patients with the following problems were excluded from the study:

- Diabetes mellitus
- Uncontrolled hypertension (systolic blood pressure > 180mmHg)
- Known causes of kidney diseases such as chronic pyelonephritis, autosomal dominant polycystic kidney disease, systemic lupus erythematosus, etc.
- Serum creatinine >250μmol/l

3.3 Investigations

3.3.1 Urine

Standard urine dipstick tests were done in all patients at the initial screening by using UriCheck 9 (RapiMed Diagnostics, Sekunjalo Health Care, Sandton, South Africa). Those with positive test for proteinuria proceeded to 24 hour urine collection to quantify the amount of protein in the urine which was done at the Chemistry laboratory, King Edward VIII Hospital, by using a timed endpoint method . Those with negative dipstick tests who agreed to further screening underwent urine analysis for microalbumin by using Micral-Test strips (Roche, Laval, Quebéc, Canada). Patients with two positive tests for microalbumin at least a month apart were selected as having persistent microalbuminuria and were asked to do 24 hour urine collection for quantification of microalbumin by the same timed endpoint method mentioned above. The confounding factors for microalbuminuria such as fever, heavy exercise, cardiac failure, hyperglycaemia, uncontrolled hypertension, acute illness, urinary tract infection and prostatic disease had been looked for and were excluded.

Based on the degree of proteinuria, patients were divided into 2 groups; those with urinary protein <1gm/24hour, including microalbuminuric patients and those with >1 gm/24hour of urine protein. The former group was randomised according to the randomization table and patients were either put on Perindopril (ACEI) or no treatment (Control). Initially 2 mg of Perindopril was used and then titrated subsequently to 4- 6mg/day as tolerated. All patients with >1 gm/24hour of proteinuria were treated with Perindopril and were followed-up long term.

Twenty-four hour urinary protein measurement was repeated after every 3 to 6 months in all patients. Patients in this study were not on anti-retroviral therapy during the study period. None of them had used heroin or any illicit drugs in the past.

3.3.2 Blood

Blood samples were taken from the patients and processed by the Chemistry Laboratory (King Edward VIII hospital) for the following tests:

- Urea and electrolytes (at entry to the study and three monthly thereafter ;serum creatinine was measured by using a modified rate Jaffe′ method)
- Blood glucose (random)
- CD4 count (by Flow cytometry ; at the entry and then 9 to 12 monthly)
- Antinuclear factor, Wasserman's reaction, Hepatitis B and C serology

3.3.3 Renal biopsy

All patients who met the inclusion criteria underwent percutaneous renal biopsy under the ultrasound guidance, using a disposable trucut needle, after the biopsy site was

adequately anaesthetised with 2% lignocaine. All the kidney tissue samples were immediately immersed into the appropriate fixatives and were sent to the Anatomical Pathology Laboratory at the Inkosi Albert Luthuli Central Hospital. Light microscopy was done in all samples. The stains included Haematoxylin and Eosin, Periodic acid Shiff, methanamine silver, Jones and Masson's trichome. Nine cases had immunofluorescence (IMF) studies done for IgA, IgG, IgM,Complement C1q, C3 and C4. (IMF studies were not done on 21 samples which were submitted at the initial part of the study. At that time, the Department of Anatomical Pathology made the ruling not to do IMF studies on samples from HIV-positive patients due to the possible hazards to the staff). Electron microscopy was performed in 6 patients (not done in the others due to technical problems with the EM machine). All biopsy samples were examined by the staff at Anatomical Pathology Department, and thereafter reviewed by a single pathologist.

3.3.4 GFR/Creatinine clearance

In terms of renal function, GFR (glomerular filtration rate) was estimated by using the Modification of Diet in Renal Disease (MDRD) equation and by calculating the creatinine clearance based on 24 hour urine samples and serum creatinine levels at study entry. Creatinine clearance was then calculated 3 monthly by using the MDRD equation as follows:

$$GFR = 186 \times SCr^{-1.154} \times Age^{-0.203} \times 0.742 \text{ (if female)} \times 1.21 \text{ (if black)}$$

[Peterson *et al.*, 1995]

3.4 Statistical Analysis

Wilcoxon test was used for statistical analysis of paired data from the same patients at the entry and end of follow-up. Mann-Whitney U-test was used for statistical analysis of unpaired data between the patients of different groups. Probability values of less than 0.05 were considered significant. Survival function analysis was done by using Kaplan-Meier survival function estimates. The non-parametric Spearman *r* test was used to determine correlations. The interval-scaled variables (initial and final levels of serum creatinine, Cr Cl and urine protein) of treated and control groups were compared using the Wilcoxon signed ranks test. To evaluate the effect of follow-up time, repeated measures ANOVA test was used which looked for the time effect on the data independent of group.

CHAPTER 4

RESULTS

4.1 Demographics of study population

A total of 615 patients were screened between April 2002 and September 2004. Six hundred and three (98%) of them were Black Africans, 9 (1.5 %) Indian, 2 (0.3%) White and 1(0.2 %) of mixed race origin. There were 156 (25 %) males and 459 (75 %) females. Mean age was 27 (range 13 – 63 years). Fifteen of them (2.4 %) were in-patients and the remaining 600 (97.6 %) were out-patients. Table 2 further defines the patient population.

Table 2. Characteristics of patients evaluated for proteinuria

Characteristics	**Normoalbuminuric**	**Microalbuminuric UP 0.03-0.3gm/24hours**	**Macroalbuminuric (Proteinuric) UP >0.3gm/24hours**	***P* value** *"♠† ‡
Demographics				
No. of patients	570	7	23 + (*15#)*	
Age *years* mean(range)	27(13-63)	32(21-40)	32(17-55)	
Sex (M/F)	139/431(24.4% /75.6%)	2/5 (29% / 71%)	10/13 (43% / 57%)	
Race – Black	559(98%)	7(100%)	22(96%)	
- Indian	9(1.6%)	0	0	
- White	2(0.3%)	0	0	
- Mixed	0	0	1	
Clinical				
BP mean *mmHg*	117/70	121/81	120/74	
Peripheral oedema (%)	0	0	61	
Initial CD4 mean	"163±34.6!!	"216±32.7♠	264±46!!♠	"0.3762 !!0.0897 ♠0.5512
Initial urine protein *gm/24hours* mean	Not done	0.14†	4.92†	†0.0138
Initial serum creatinine *µmol/l* mean	60	66•	125•	•0.0071
Initial Creatinine Clearance *ml/min* mean	Not done	139‡	77‡	‡0.0009
HIVAN	-	6(86%)	19(83%)	

refused /defaulted; excluded from further analysis; HIVAN=HIV-associated nephropathy; BP=blood pressure

Altogether, 30 patients were recruited for the biopsy arm of the study. There were variable follow-up periods ranging from 11 days to 667 days with a mean of 304 days.

4.2 Proteinuria

Thirty eight patients (6.0%) were found to have proteinuria (proteinuria is defined as having 1+ or more of protein on standard urine dipstick testing). Fifteen of them (39 %) were in-patients and 23 (61 %) were out-patients.. Five patients refused renal biopsy, and 10 patients were lost to follow up before the biopsy was performed. Therefore, a total of 15 patients with proteinuria were excluded from the study. In total, 23 proteinuric patients (15 in-patients and 8 out-patients) underwent renal biopsy. Quantification of proteinuria revealed mean of 6 gm/24hour (range 0.47 - 24.75) on study entry.

Of the 30 patients, 11 had proteinuria of < 1 gm/24hour, whereas 19 patients had >1 gm/24hour proteinuria at initial presentation. Tables 3 and 4 illustrate the characteristics of these patients.

Table 3. Characteristics of patients with proteinuria < 1 gm/24hr

Patient	Age	Gender	Race	CD4	Renal biopsy	UP (gm/24hr)
Controls						
1	28	F	B	183	HIVAN	0.9
2	40	F	B	39	IN	0.48
3	30	F	B	239	IN	0.13
4	21	F	B	137	HIVAN	0.07
5	40	M	B	199	HIVAN+IN	0.23
ACEI Group						
6	34	F	B	611	IN	0.47
7	29	F	B	3	HIVAN	0.94
8	26	F	B	204	HIVAN+IN	0.06
9	34	F	B	246	HIVAN	0.30
10	43	M	B	114	HIVAN+Mem GN	0.04
11	27	F	B	370	HIVAN	0.17

UP = Urine Protein; HIVAN = HIV-associated nephropathy; IN= Interstitial Nephritis
Mem GN= Membranous glomerolonephritis

Table 4. Characteristics of patients with proteinuria > 1gm/24hr

Patient	Age	Gender	Race	CD4	Renal biopsy	UP (gm/24hr)
ACEI Group						
1	25	M	B	392	HIVAN	1.14
2	30	F	B	213	HIVAN	1.35
3	29	M	B	90	HIVAN	1.38
4	42	M	B	130	HIVAN	2.23
5	29	F	B	145	HIVAN	4.47
6	24	F	B	335	HIVAN	3.61
7	55	M	B	402	HIVAN	4.92
8	32	F	B	145	HIVAN	5.75
9	26	F	B	-	HIVAN	5.19
10	26	M	B	586	HIVAN	6.33
11	39	M	B	-	HIVAN	6.83
12	21	F	B	130	HIVAN	9.21
13	42	M	C	210	MPGN	5.45
14	42	M	B	-	HIVAN	11.64
15	24	F	B	-	HIVAN+Mem GN	11.15
16	29	M	B	641	MPGN	12.98
17	39	F	B	159	HIVAN+Mem GN	14.0
18	29	M	B	331	HIVAN+Mem GN	24.75
19	17	F	B	-	HIVAN	2.9

UP = Urine Protein ; Mem GN = Membranous Glomerulonephritis
MPGN= Membranoproliferative glomerulonephritis

CD4 counts were not available in 5 patients because blood specimens did not reach the laboratory. All patients defaulted before another samples could be taken. From the <1gm/24hour proteinuric group, 6 were randomly put on Perindopril (ACEI) and 5 were followed up as a control group on no treatment. One patient from the treatment group (patient number 9, Table 3 and Table 5) lived at a great distance from the hospital and could not come regularly for her monthly medications. Therefore, she was excluded from further analysis in the study. At the end of follow-up, the ACEI-treated group showed stable proteinuria, whereas the control group revealed non-significant increase in proteinuria ($p = 0.357$) (Figure 11).

Table 5. Outcomes of patients with proteinuria < 1gm/24hr

Patient	Se Cr (µmol/l)		Cr Cl		UP(gm/24hr)		Follow up (days)	Outcome	Remark
	Entry	Final	Entry	Final	Entry	Final			
Controls									
1	69	153	80	45	0.9	0.95	220	defaulted	
2	85	147	83	44	0.48	0.51	638	alive	
3	37	43	229	191	0.13	0.10	561	defaulted	
4	61	74	139	110	0.07	0.08	542	defaulted	
5	111	120	82	75	0.23	0.94	560	alive	also received Azathioprine for AIH
ACEI group									
6	73	71	102	105	0.47	0.38	367	Deceased	Died from meningitis
7	77	64	99	122	0.94	0.74	154	Deceased	Died from PCP
8	65	64	124	125	0.06	-	105	Deceased	Died from PCP
†9	62	88	123	82	0.30	0.78	498	defaulted	stays far, treatment interrupted
10	70	77	138	116	0.04	0.02	667	alive	
11	58	47	140	177	0.17	0.13	323	alive	

†excluded from the study; Se Cr=Serum Creatinine; UP=Urine protein; AIH=Autoimmune Hepatitis; PCP=Pneumocystis carinii pneumonia

Table 6. Outcomes of patients with proteinuria > 1gm/24hr

Patient	Se Cr (μmol/l)		*P* value	Cr Cl		*P* value	UP(gm/24hr)		*P* value	Outcome
	entry	end		entry	end		entry	end		
1	72	73		148	145		1.14	0.61		Defaulted
2	156	147		43	47		1.35	-		Died from PCP
3	101	86		98	118		1.38	0.34		Alive
4	80	80		118	118		2.23	1.39		Alive
5	172	170		39	40		4.47	-		Died from PCP at 2/12
†6	112	573‡		76	10‡		3.61	-		Rx stopped after 1/12 because of ↑K+, died from disseminated TB ‡value at time of death (at 3/12)
7	103	97		84	90		4.92	1.27		Alive
8	199	130		32	51		5.75	3.42		Died from CVA
9	155	76		45	103		5.19	-		Died from Pneumonia
10	90	-		114	-		6.33	-		Defaulted
11	146	-		60	-		6.83	-		Defaulted
12	178	158		40	46		9.21	8.0		Defaulted
†13	193	119/ 520*		35	68/ 11*		5.45	-		Defaulted after 2/12 *value on readmission 2/12 later on no Rx
14	213	160		38	53		11.64	10.0		Defaulted
15	63	56		130	149		11.15	8.5		Alive
16	198	218		45	40		12.98	-		Defaulted
17	181	88		35	80		14.0	3.66		Defaulted
18	78	52		132	208		24.75	0.83		Alive
19	82	69		103	125		2.9	-		Defaulted
Mean	*136*	*111.2*	*0.334*	*76.7*	*94.2*	*0.111*	*7.12*	*3.8*	*0.003*	

† excluded from the study; PCP=Pneumocystis carinii pneumonia; CVA=Cerebrovascular accident

Patients 10 and 11 did not have final creatinines as they defaulted before the bloods were taken. All patients with proteinuria >1gm/24hour were treated with Perindopril. The anti-proteinuric effect of ACEI was clearly seen among this group of patients with significant proteinuria. Twenty-four hour urinary protein excretion significantly dropped from the base line value at the end of follow-up ($p = 0.003$) (Figure 14). The degree of anti-proteinuric effect was found to be correlated with the duration of follow-up as shown below.

[change in urinary protein = -0.017 x follow-up time (days) + 1.599]

All patients who were treated with Perindopril tolerated the ACEI well except for one patient with HIVAN (patient number 6, Table 6) from the >1gm/24hour urine proteinuria group developed hyperkalaemia and ACEI treatment was stopped after one month. Her renal function deteriorated and she died 2 months later with a diagnosis of disseminated tuberculosis. Serum creatinine at the time of death was 573 μmol/l. Another patient from the same group with MPGN (patient number13,Table 6) defaulted treatment after 2 months and was readmitted with severely deranged renal function (Serum creatinine 520 μmol/l) 2 months after discontinuation of ACEI therapy. Both patients were excluded from further analysis.

4.3 Microalbuminuria

Microalbuminuria was tested in 90 patients who agreed to take part in the study. Thirty two patients (35.6%) had microalbuminuria; however, persistent microalbuminuria was found in only 7 patients (7.8%). All patients with persistent microalbuminuria agreed to participate further in the study. Altogether, 30 patients (23 with proteinuria and 7 with microalbuminuria) were recruited. Characteristics of patients with microalbuminuria were shown in Table 2 and Table 7.

Table 7. Characteristics of patients with microalbuminuria

Patient	Age (yrs)	Race	Gender	CD4	Se creatinine (µmol/l)	Cr Clearance (ml/min) [Measured]	Cr Clearance (ml/min/1.73 m²) [Calculated]	Hepatitis serology		Urine protein (gm/24hr)	Renal biopsy
								B	C		
1	30	B	F	239	37	188	188	-	-	0.13	IN
2	26	B	F	204	65	63	123	-	NA	0.06	HIVAN+IN
3	34	B	F	246	62	95	123	-	-	0.30	HIVAN
4	40	B	M	199	93	78	100	-	-	0.23	HIVAN+IN
5	43	B	M	114	70	94	137	-	-	0.04	HIVAN+Mem
6	27	B	F	379	68	104	116	-	-	0.17	GN
7	21	B	F	137	61	130	138	-	-	0.07	HIVAN
											HIVAN

IN=Interstitial Nephritis ; Mem GN=Membranous glomerulonephritis ; NA= not available

Table 2 compares the characteristics of all microalbuminuric and proteinuric patients. There were no statistically significant differences in mean age, mean blood pressure, initial mean CD4 count between the 2 groups except for the higher female to male ratio in microalbuminuric patients. Peripheral oedema was absent in all patients with microalbuminuria, whereas it was present in the majority (61%) of proteinuric patients. Initial 24 hour urinary protein and serum creatinine were significantly higher in the proteinuric group (p= 0.0138 and 0.0071 respectively). Initial Cr Cl was significantly lower in the proteinuric group (p= 0.0009). However, HIVAN was found to be equally present in both groups (86% and 83%).

4.4 CD4 count

The mean CD4 count at the screening of all 615 patients was 196 (range 0-1088)/mm³. Patients with proteinuria had a mean CD4 count of 264 (range 3 – 611) and those with persistent microalbuminuria had a mean CD4 count of 216 (range 114 - 379); (Table

2). Patients with normoalbuminuria had a mean CD4 count of 163 (range 0 – 1088). Mean CD4 count of patients with HIVAN was 232 (range 3 – 586).

4.5 Creatinine Clearance

Creatinine clearance (Cr Cl) was both measured and calculated at entry to the study to assess the correlation. Both correlated well in patients with any degree of proteinuria (Correlation Coefficient = 0.802). Mean Cr Cl at entry was significantly lower in proteinuric groups, compared to the microalbuminuric group ($p = 0.0009$) (Table 2). With ACEI therapy in patients with < 1gm/24hour of proteinuria, there was non-significant improvement in Cr Cl at the end of follow-up period (p=0.405), however, it was significantly worsened in the controls group (p=0.042) (Figure 13). Cr Cl was better preserved among the patients with >1 gm/24hour of proteinuria who received ACEI, although it was not statistically significant (p=0.111) (Figure 16).

Table 8. Characteristics of patients with proteinuria < 1gm/24 hr by treatment group

Characteristics	Control ($N = 5$)	ACEI Group ($N = 5$)	*P value
Age (yrs) *mean*	31.8	31.8	*1.0000*
Entry CD4 *mean*	159 ± 34.2	260 ± 106.2	*0.3920*
Final CD4 *mean*	104 ± 28.6	259 ± 90.7	*0.1148*
*P value	*0.1837*	*0.8760*	
Entry Se Cr (µmol/l) *mean*	72.6 ± 12.3	68.6 ± 3.2	*0.7826*
Final Se Cr (µmol/l) *mean*	107.4 ± 21.2	64.6 ± 5	*0.1314*
*P value	*0.047*	*0.3327*	
Entry Cr Cl (ml/min)*mean*	122.6 ± 28.8	120.6 ± 8.6	*0.9455*
Final Cr Cl (ml/min) *mean*	93 ± 27.3	129 ± 12.4	*0.2964*
*P value	*0.042*	*0.405*	
Entry UP(gm/24hr) *mean*	0.36 ± 0.15	0.34 ± 0.16	*0.9118*
Final UP (gm/24hr) *mean*	0.52 ± 0.19	0.32 ± 0.15	*0.6628*
*P value	*0.3320*	*0.1182*	

**Student t test*

The effect of ACEI therapy in delaying disease progression was assessed by comparing the percentage of patients whose Cr Cl had halved at the end of the study. No patients from proteinuria of < 1gm/24 hr group on ACEI therapy had their Cr Cl halved, whereas 1 out of 5 (20%) patients from control group had this degree of declining renal function. From the >1gm group, 2 out of 19 (10.5%) patients showed halving of Cr Cl (Table 9).

Table 9 . Percentage of patients with halving of creatinine clearance at the end of study (proteinuria <1gm/24hour group vs >1gm/24hours)

Proteinuria group				Creatinine clearance halved		
				no	yes	Total
<1g	Group	ACEI	Count	5	0	5
			% within Group	100.0%	.0%	100.0%
		Control	Count	4	1	5
			% within Group	80.0%	20.0%	100.0%
	Total		Count	9	1	10
			% within Group	90.0%	10.0%	100.0%
>1g	Group	ACEI	Count	17	2	19
			% within Group	89.5%	10.5%	100.0%
	Total		Count	17	2	19
			% within Group	89.5%	10.5%	100.0%

Kaplan-Meier survival function analysis (halving of Cr Cl) was also performed in controls (5 patients) and the ACEI treated group(24 patients). More patients who had halved their Cr Cl were found in the control group which, however was not statistically significant ($p = 0.7835$). Similarly, a non-significant difference was found when comparing the 2 proteinuric groups ($p = 0.6418$) (Figure 9 and Figure 10).

Figure 9. Kaplan-Meier survival functions (halving of creatinine clearance) in control and ACEI treated groups

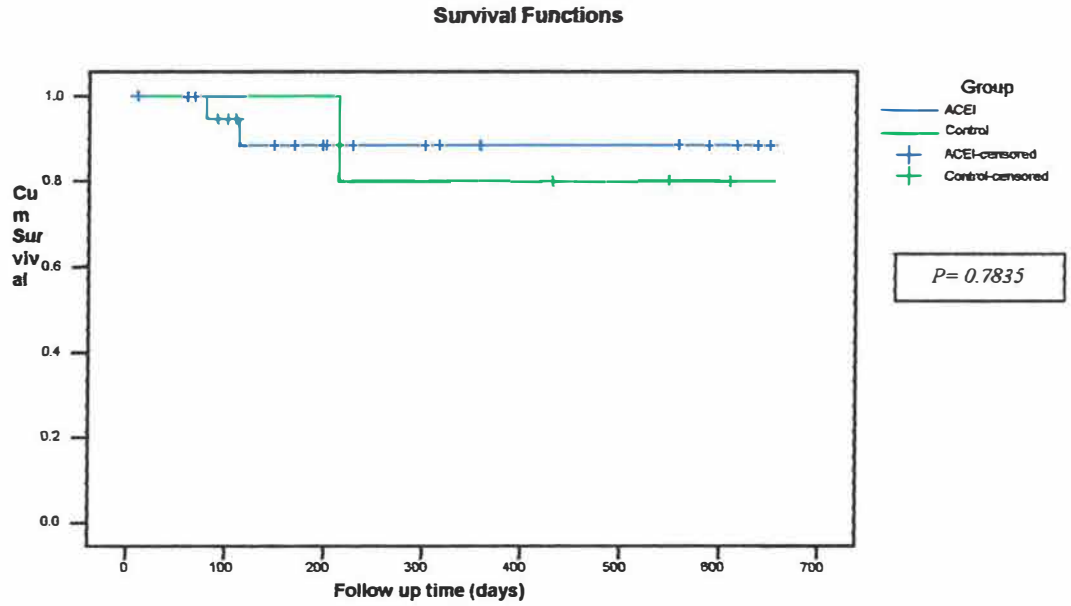
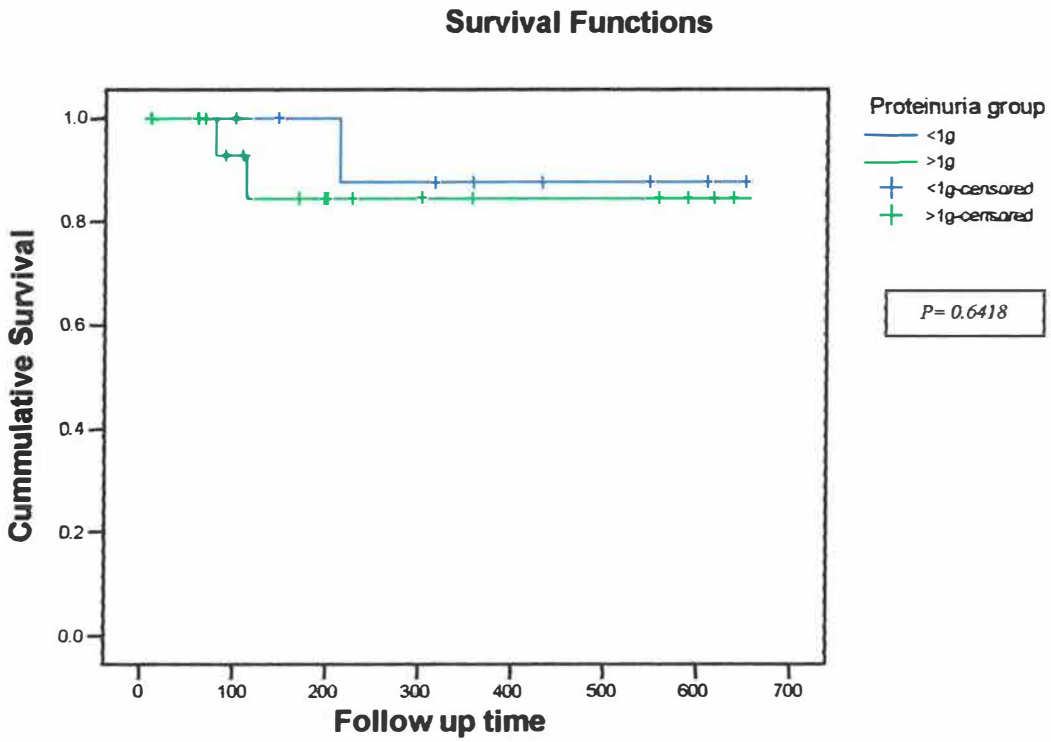


Figure 10. Kaplan-Meier survival functions (halving of creatinine clearance) in patients with proteinuria of < 1gm/24hours and >1gm/24hours



Patients with proteinuria < 1gm/24hours

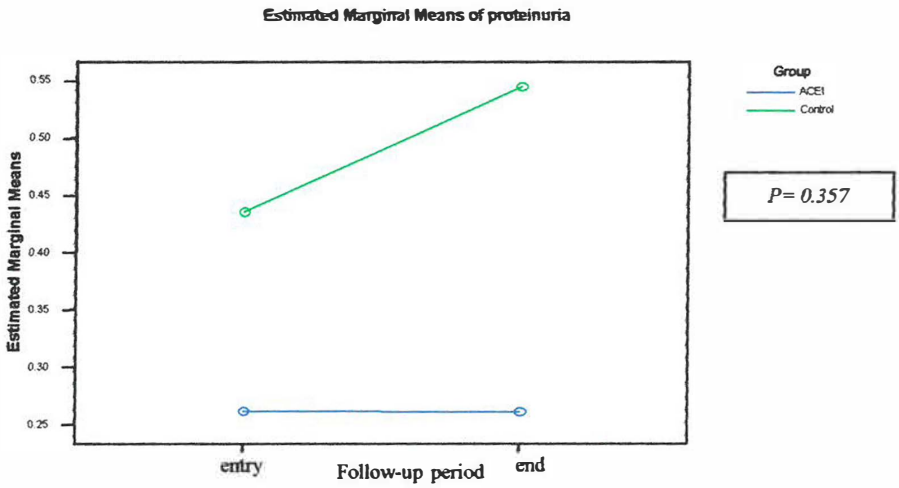


Figure 11. Change in mean proteinuria [entry and end of study]

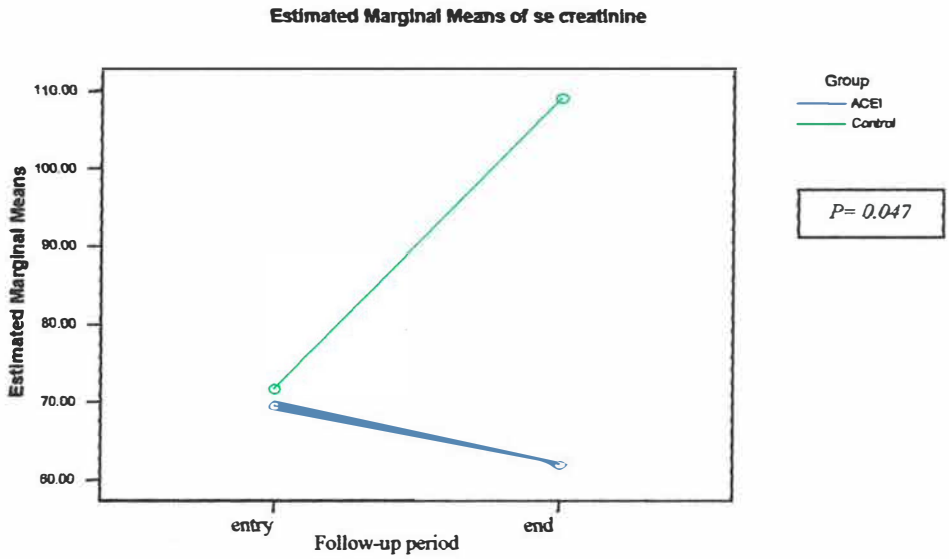


Figure 12. Change in mean serum creatinine [entry and end of study]

Patients with proteinuria < 1gm/24hours (*Continued*)

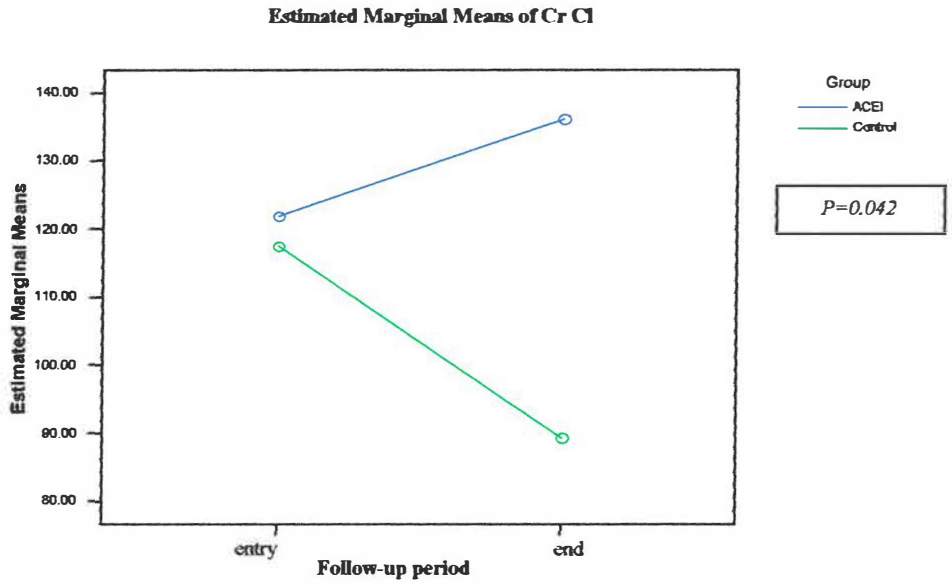


Figure 13. Change in mean creatinine clearance [entry and end of study]

Patients with proteinuria > 1gm/24hours

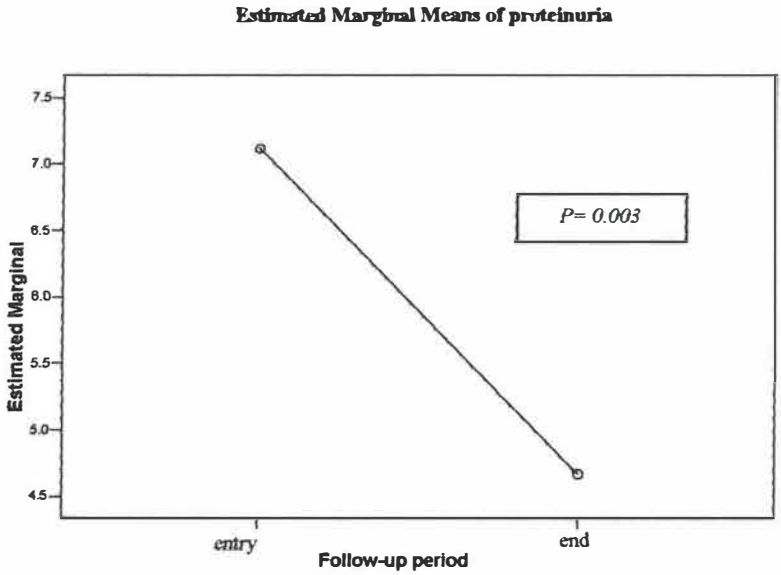


Figure 14. Change in mean proteinuria with ACEI therapy [entry and end of study]

Patients with proteinuria > 1gm/24hours

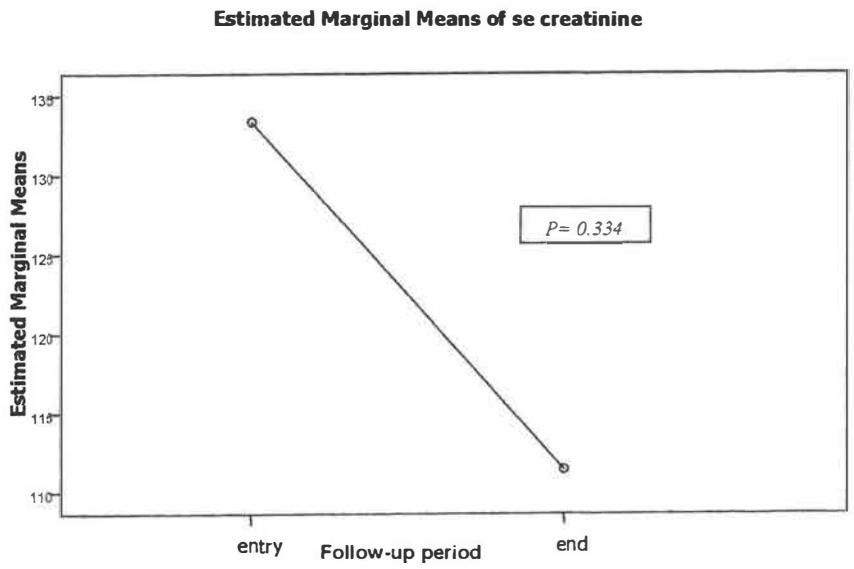


Figure 15. Change in mean serum creatinine with ACEI therapy [entry and end of study]

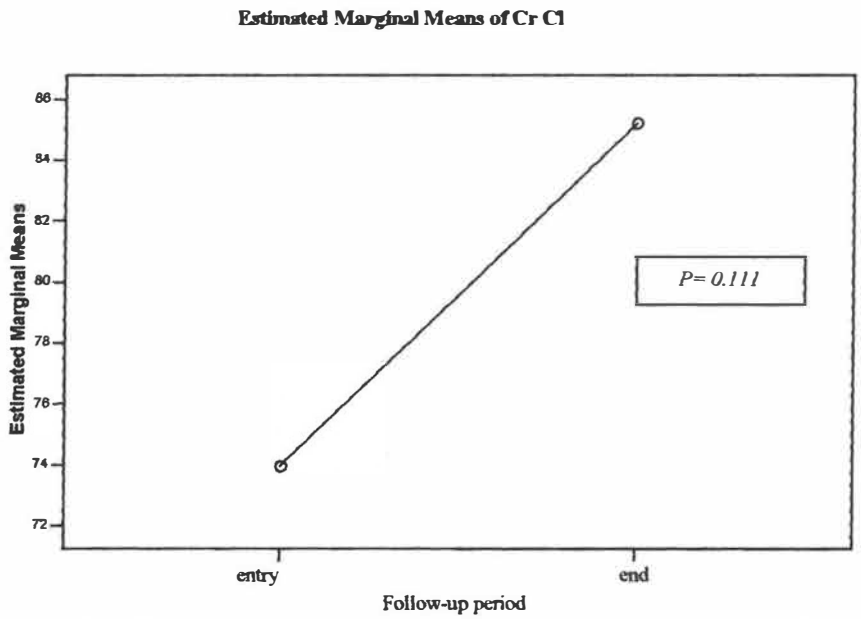
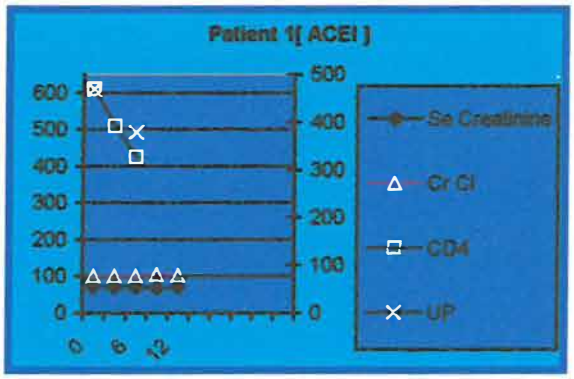
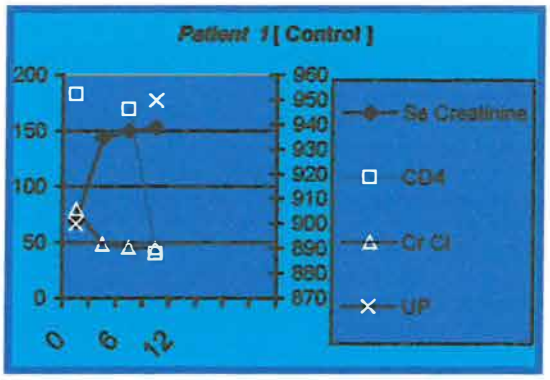


Figure 16.Change in mean creatinine clearance with ACEI therapy [entry and end of study]

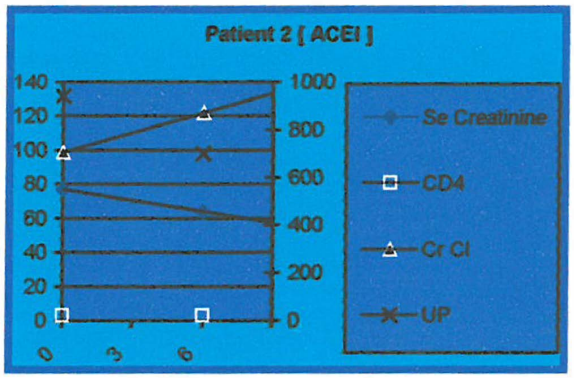
Figure 17. Variables of individual patients with proteinuria < 1gm/24hr (Controls vs ACEI therapy). Serial changes of serum creatinine, creatinine clearance (Cr Cl), and CD4 count (y axis), 24 hour urine protein (UP) (secondary y axis) with or without ACEI treatment during the follow-up period (months; x axis) are shown for each patient (controls were illustrated on the left column and treatment group on the right column for comparison). Histological diagnoses are described at the bottom of each slide. (**MA** = microalbuminuria) [Units: Se creatinine (μmol/l); Cr Cl (ml/min); CD4 (/mm³); UP (mg/24hr)]



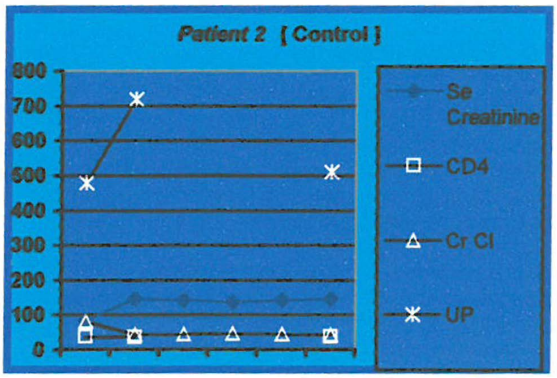
(1) Interstitial Nephritis



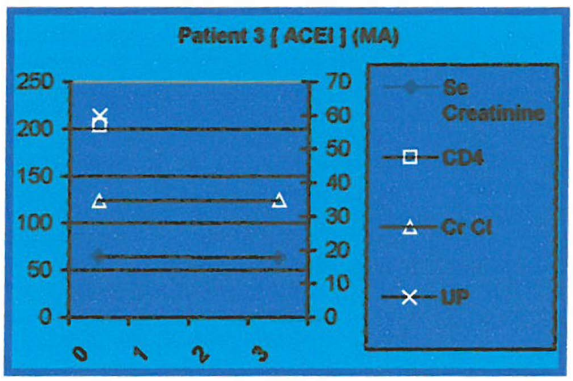
(1) HIVAN



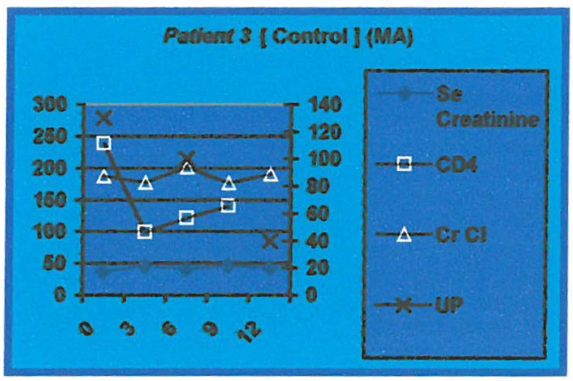
(2) HIVAN



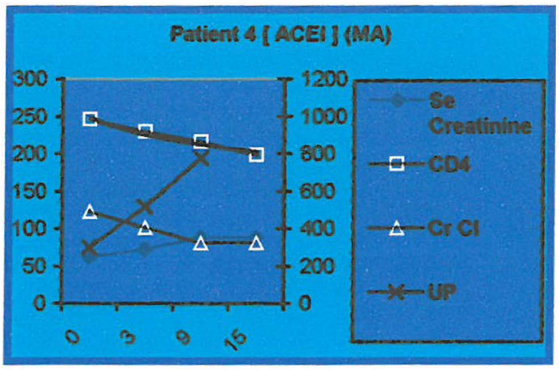
(2) Interstitial Nephritis



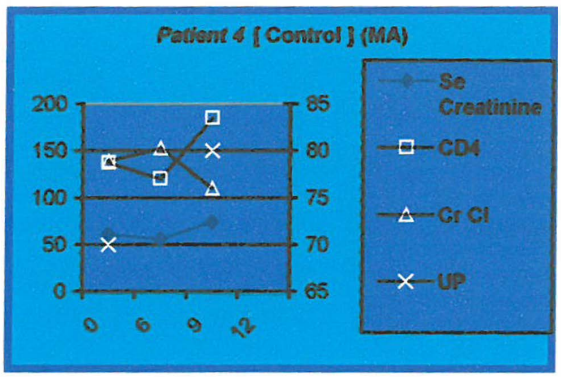
(3) HIVAN + Interstitial Nephritis



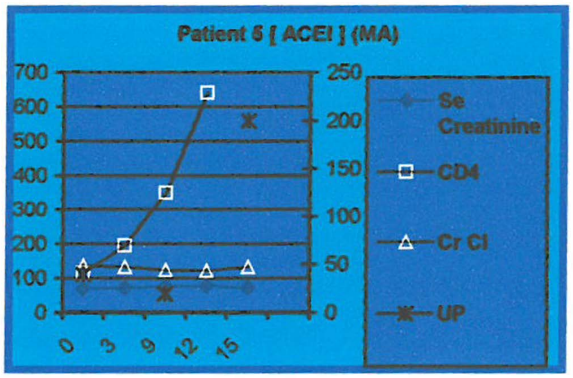
(3) Interstitial Nephritis



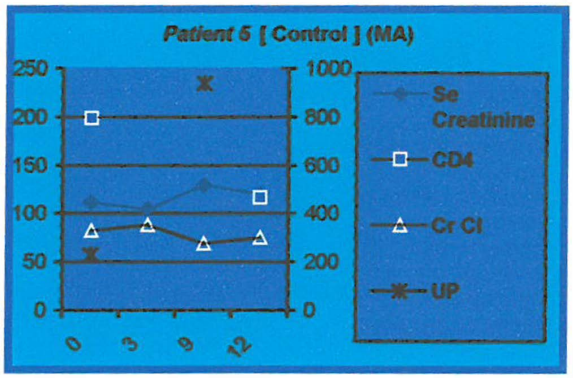
(4) HIVAN



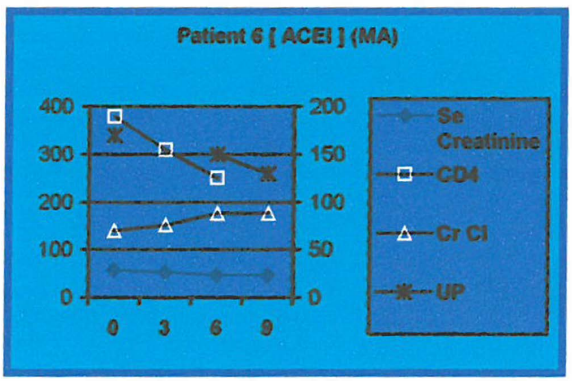
(4) HIVAN



(5) HIVAN + Interstitial Nephritis

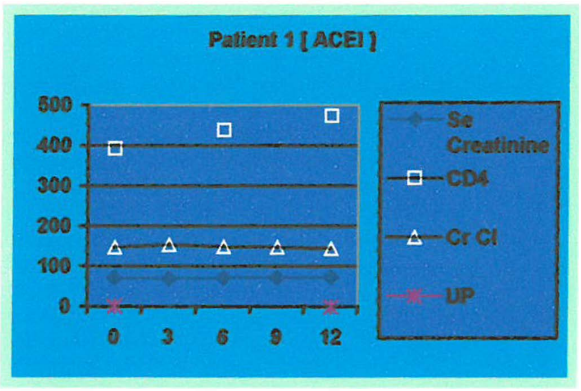


(5) HIVAN+ Interstitial Nephritis

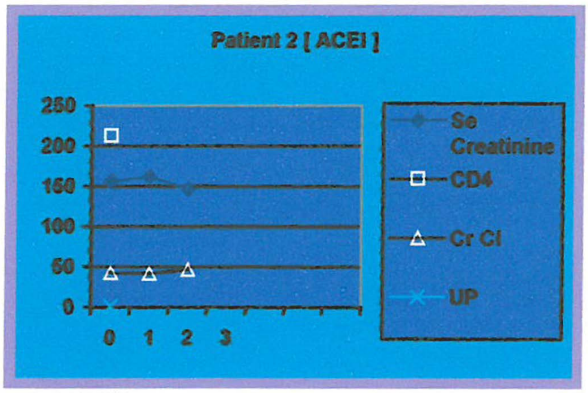


(6) HIVAN

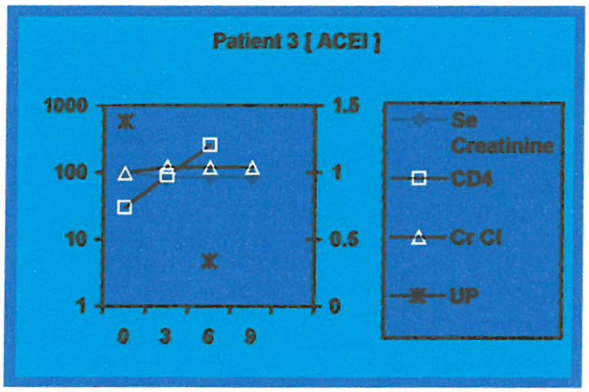
Figure 18. Variables of patients with proteinuria > 1gm/24hr (all on ACEI therapy)
Serial changes of Serum creatinine, Cr Cl, CD4 counts and 24 hour urine protein (UP) during the follow-up period are shown for each patient. Histological diagnoses are mentioned at the bottom of each slide.
[Units: Serum creatinine(μmol/l); Cr Cl(ml/min); CD4(/mm³); UP(gm/24hours)]
HIVAN=HIV-associated nephropathy; MPGN=Membranoproliferative glumerulonephritis; IN=Interstitial nephritis



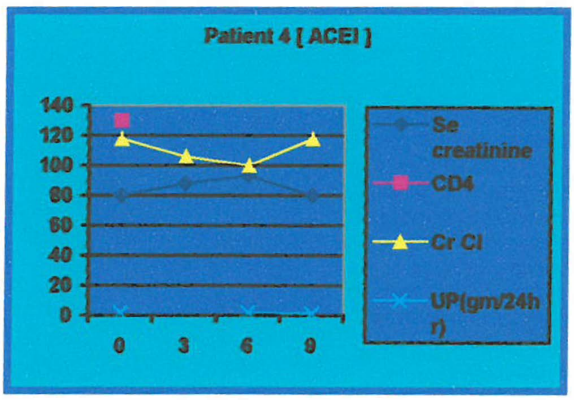
HIVAN



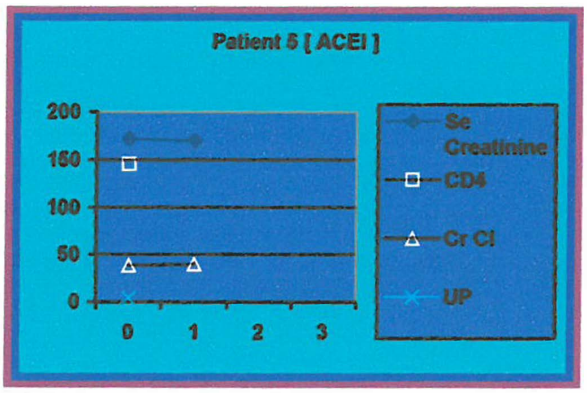
HIVAN



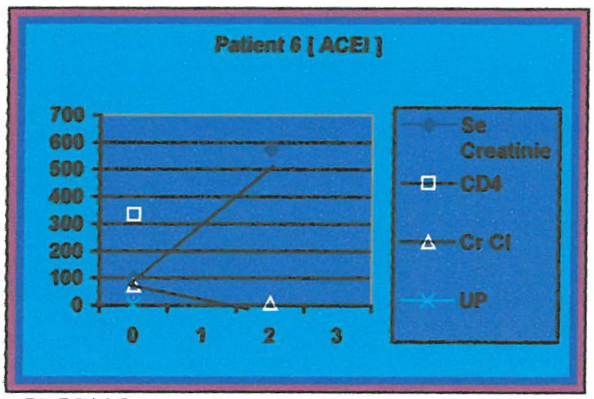
HIVAN



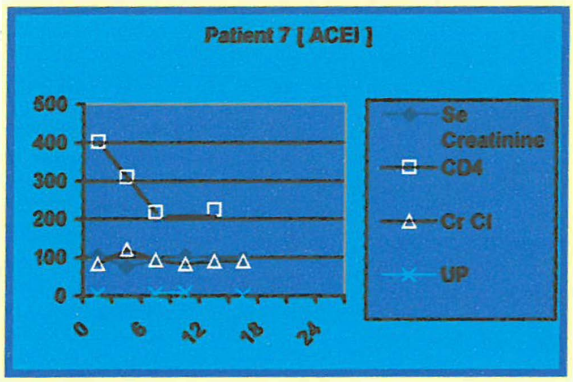
HIVAN



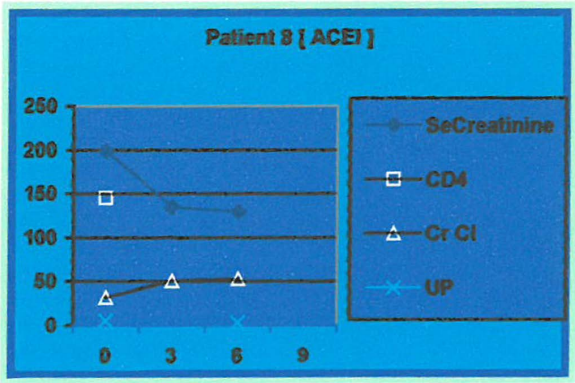
HIVAN



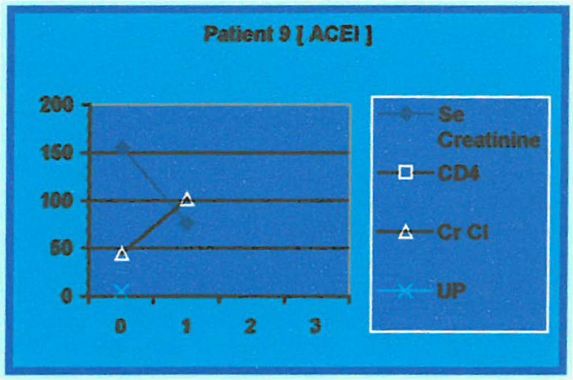
HIVAN



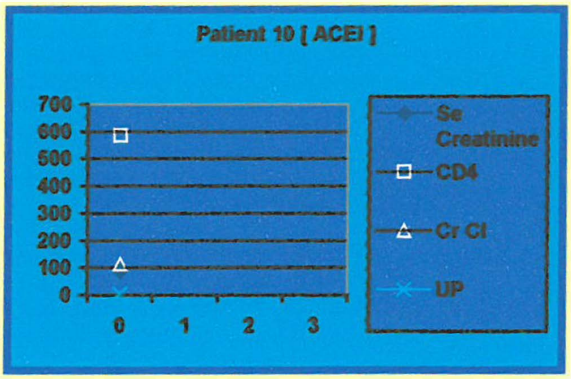
HIVAN



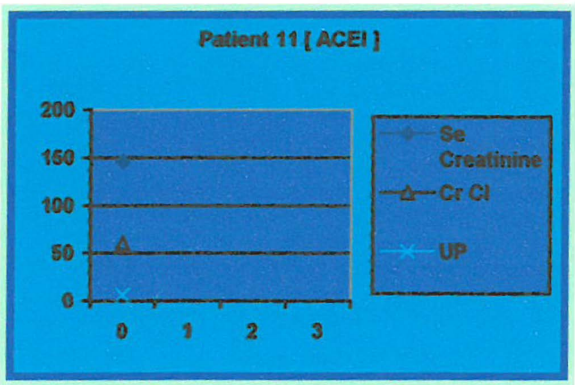
HIVAN



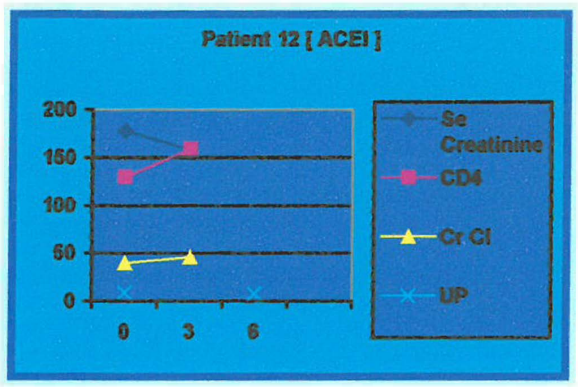
HIVAN



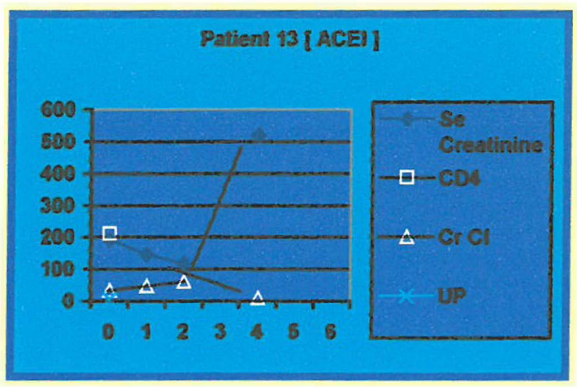
HIVAN



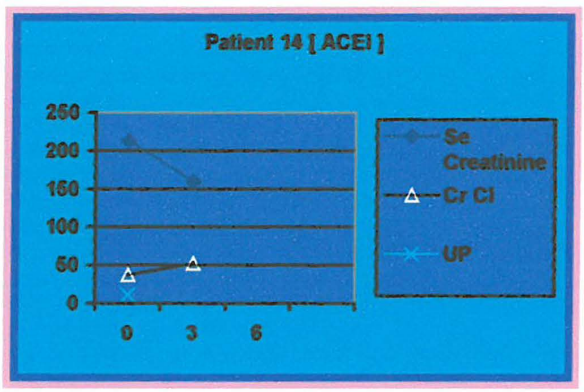
HIVAN



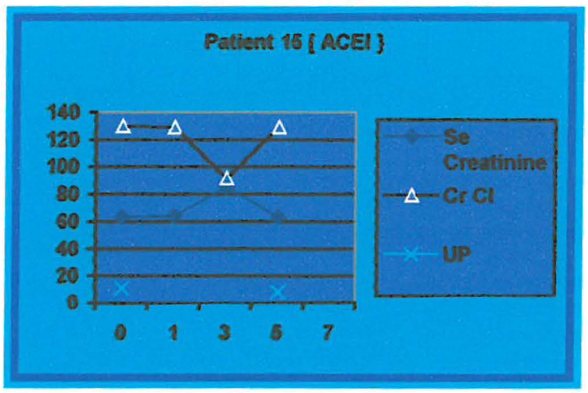
HIVAN



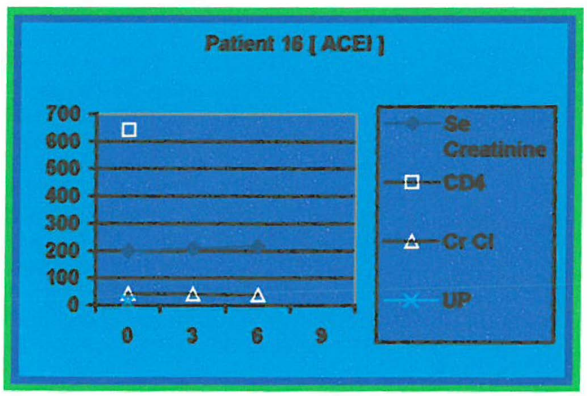
MPGN



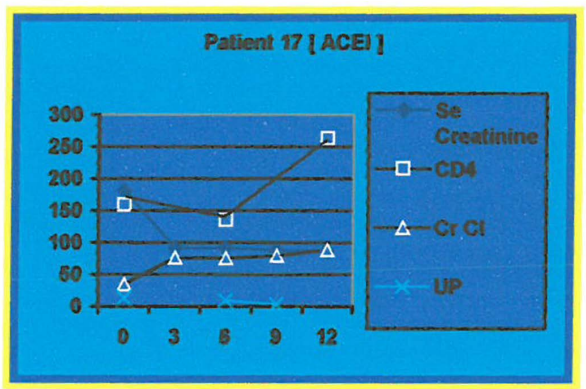
HIVAN



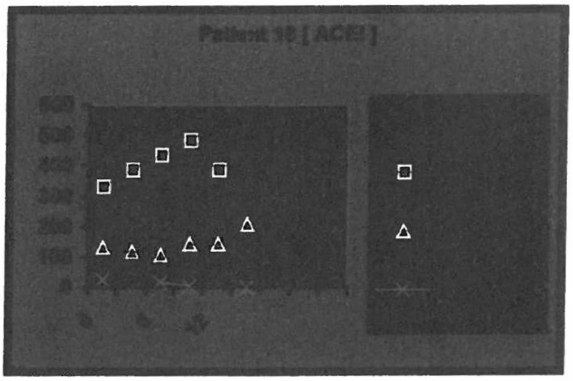
HIVAN + Membranous GN



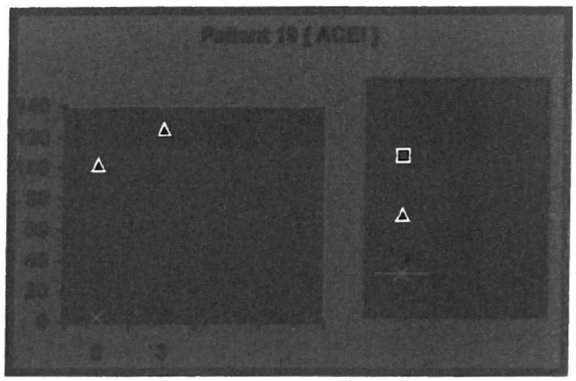
MPGN



HIVAN + Membranous GN



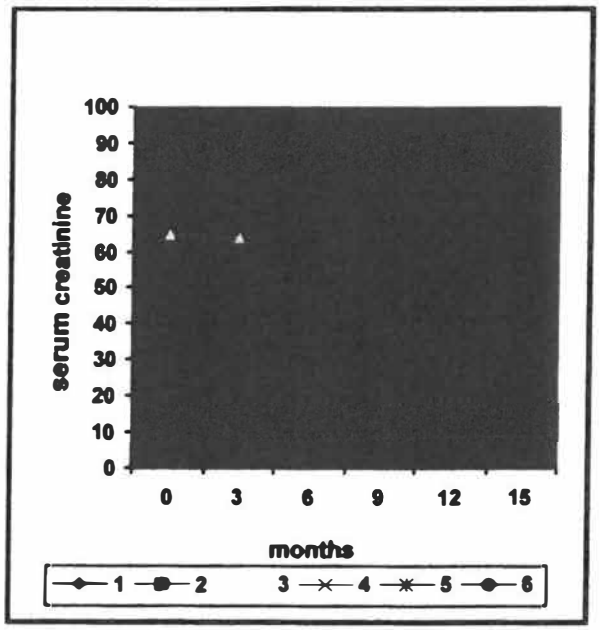
HIVAN + Membranous GN



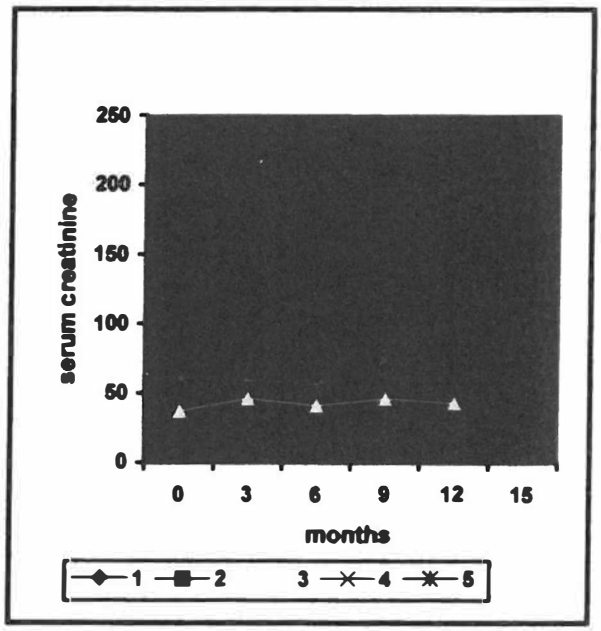
Membranous GN

Figure 19. All patients with proteinuria < 1 gm/24hr (Control vs ACEI therapy)
All patients from ACEI therapy group or Controls are shown. Serial changes of serum creatinine, Cr Cl, 24 hour urine protein and CD4 counts are plotted against the follow-up period. ACEI treated group (6 patients) are illustrated on the left and Controls (5 patients) on the right for comparison.

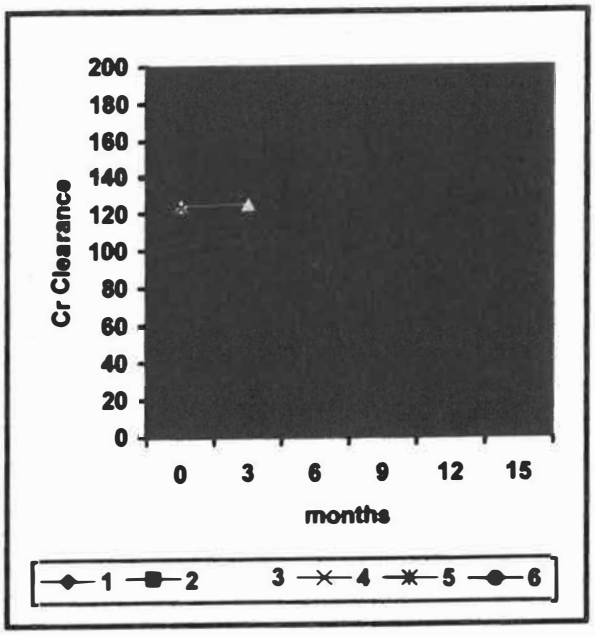
ACEI

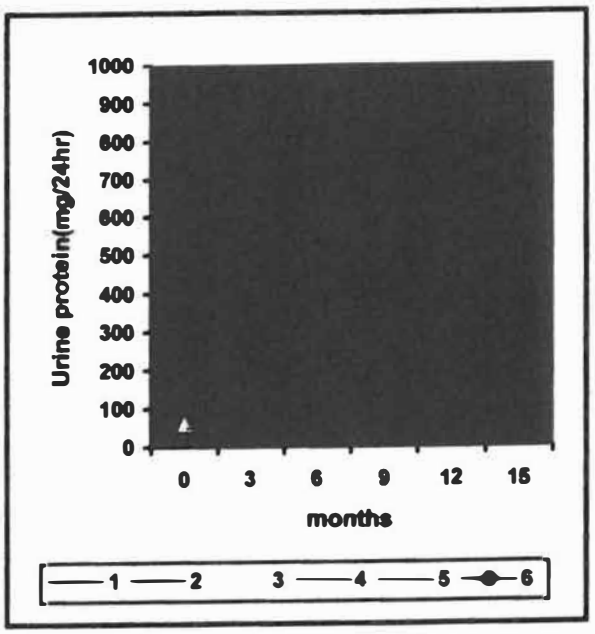


CONTROLS

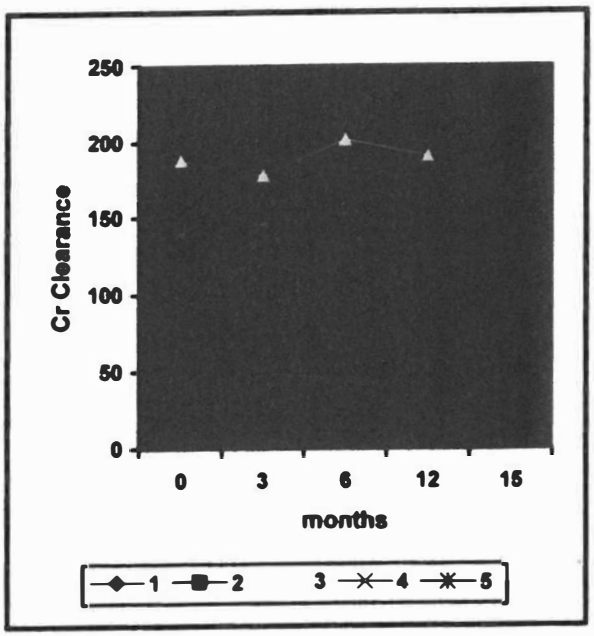


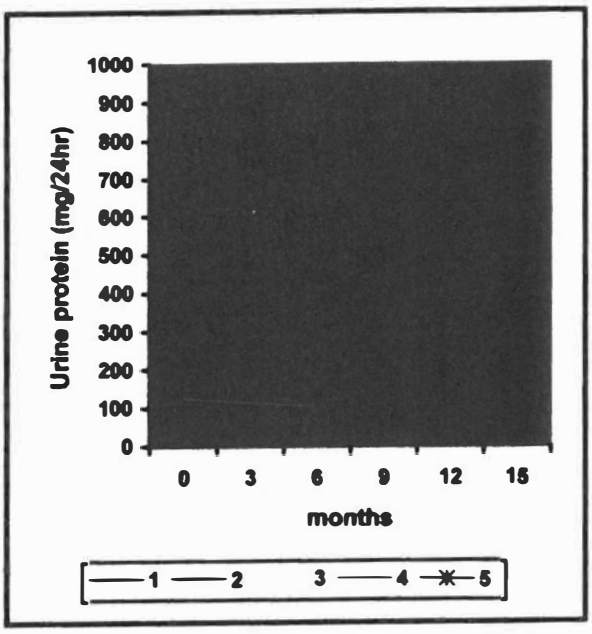
ACEI





CONTROLS





ACEI **CONTROLS**

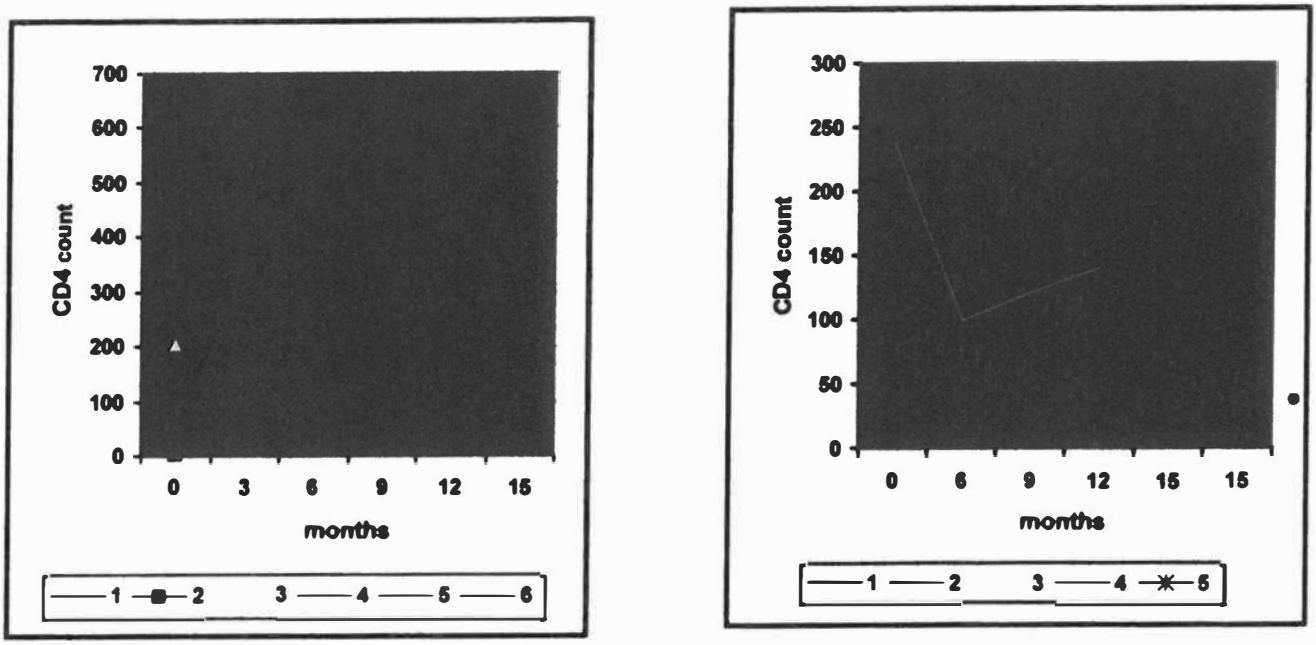
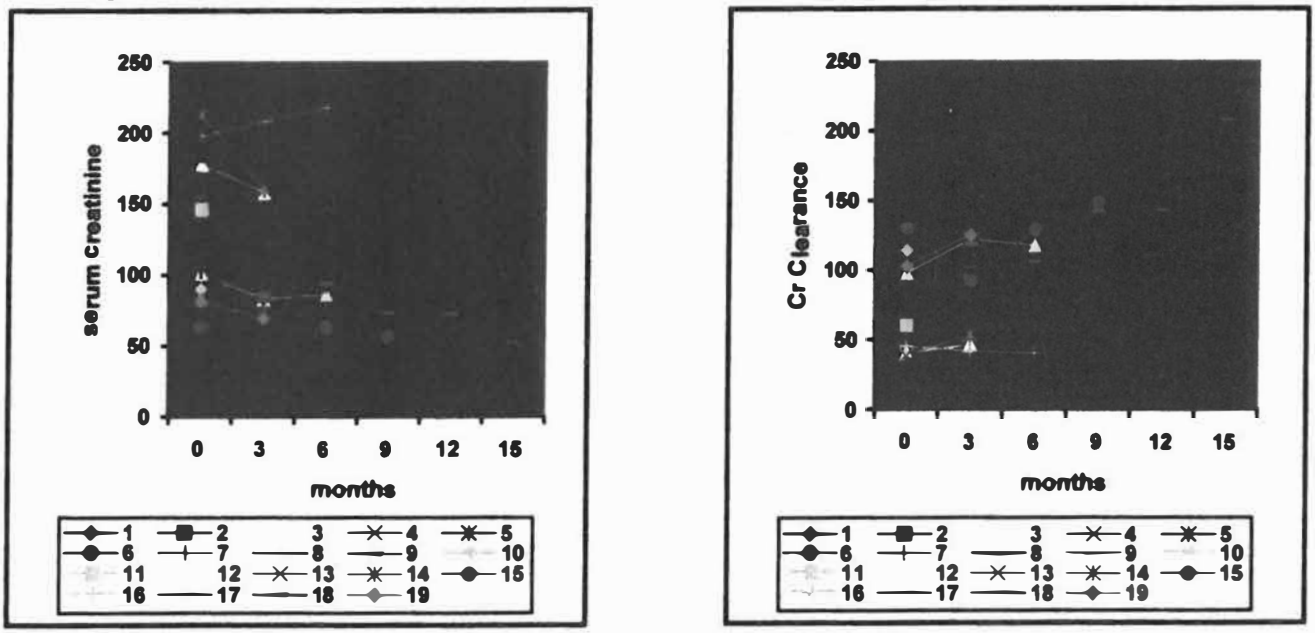
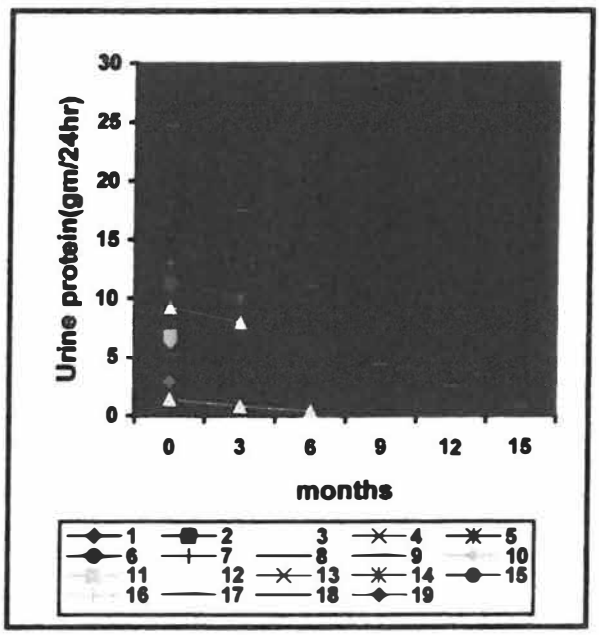
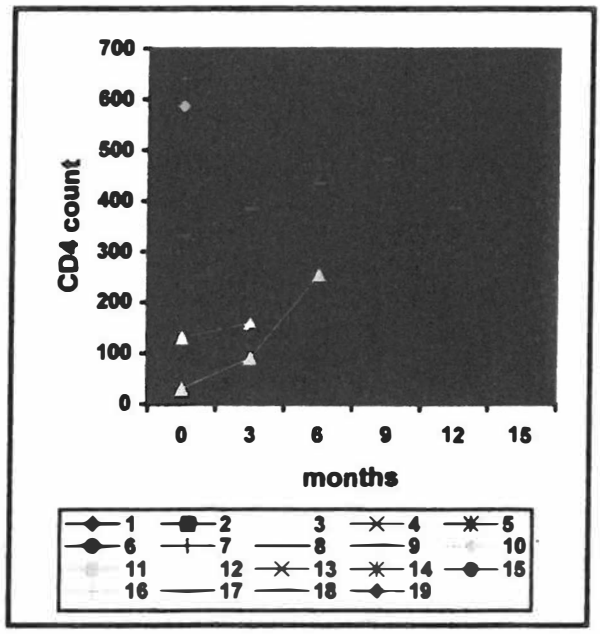


Figure 20. Patients with proteinuria > 1 gm/24hr
All 19 patients form this group were treated with ACEI. Serial changes of serum creatinine, Cr Cl, 24 hour urine protein and CD4 counts are shown against the follow-up period.



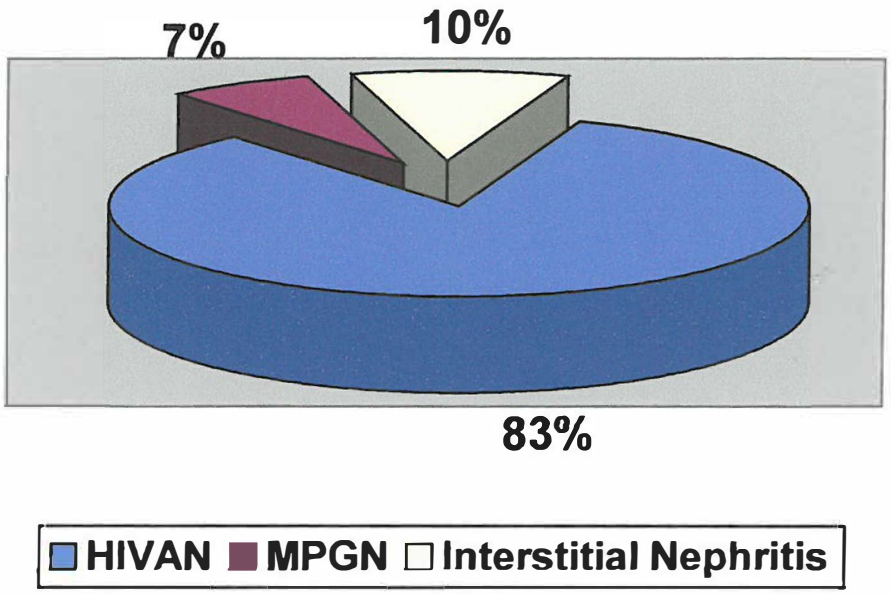




4.6 Histological types

Thirty patients with varying degree of proteinuria underwent renal biopsy. HIVAN was present in 25 (83 %) with a mean CD4 count of 232 (range 3 - 586) (Table10) (Figure 22). Of 25 patients, 6 (24%) had microalbuminuria. Altogether, 7 patients with microalbuminuria were biopsied and 6 (85.7%) showed HIVAN with a mean CD4 count of 216 (range 137-379/mm^3) (Table 7). Four patients with HIVAN had associated membranous nephropathy (Table 11) (Figure 23 and 24). Two of them were hepatitis B serology positive. Other biopsy findings included 2 (7 %) patients with membranoproliferative nephropathy and 3(10%) with interstitial nephritis (Figure 21).

Figure 21. Histological types on renal biopsy



Total HIVAN = 25 (83%)

HIVAN only = 21 (70%)

HIVAN + Membranous GN = 4 (13%)

Figure 22. Classical HIVAN (collapsing glomerulus)

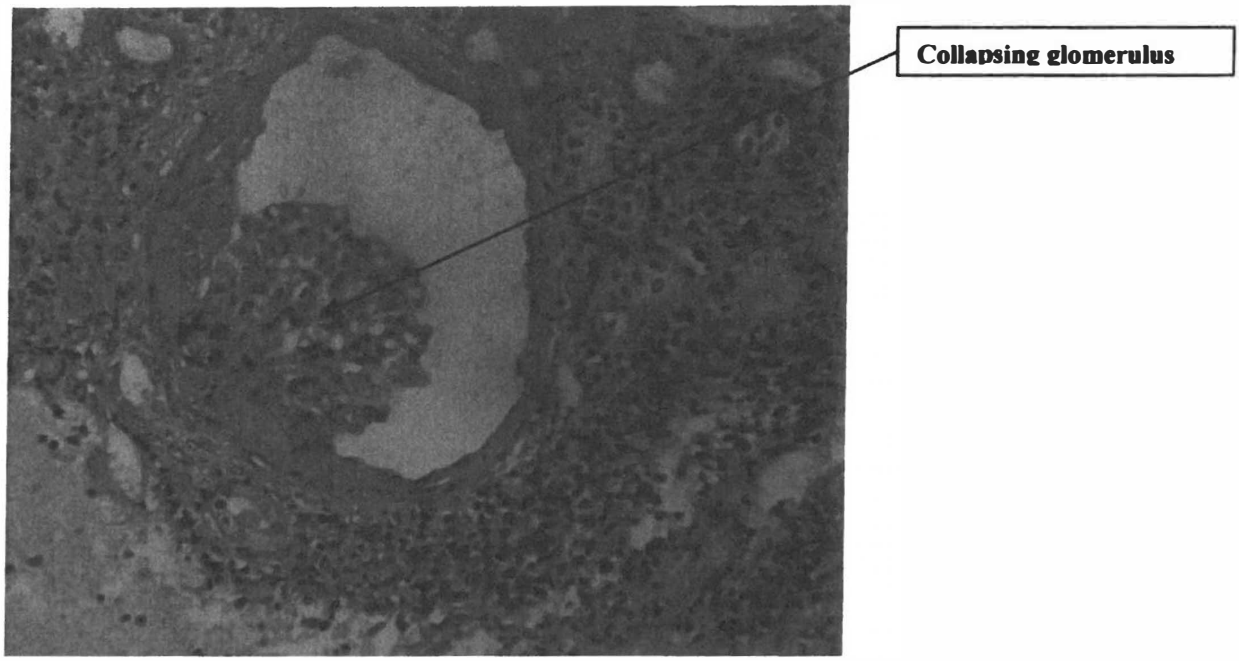


Figure 23 (a). Membranous GN combined with HIVAN (collapsing glomerulus)

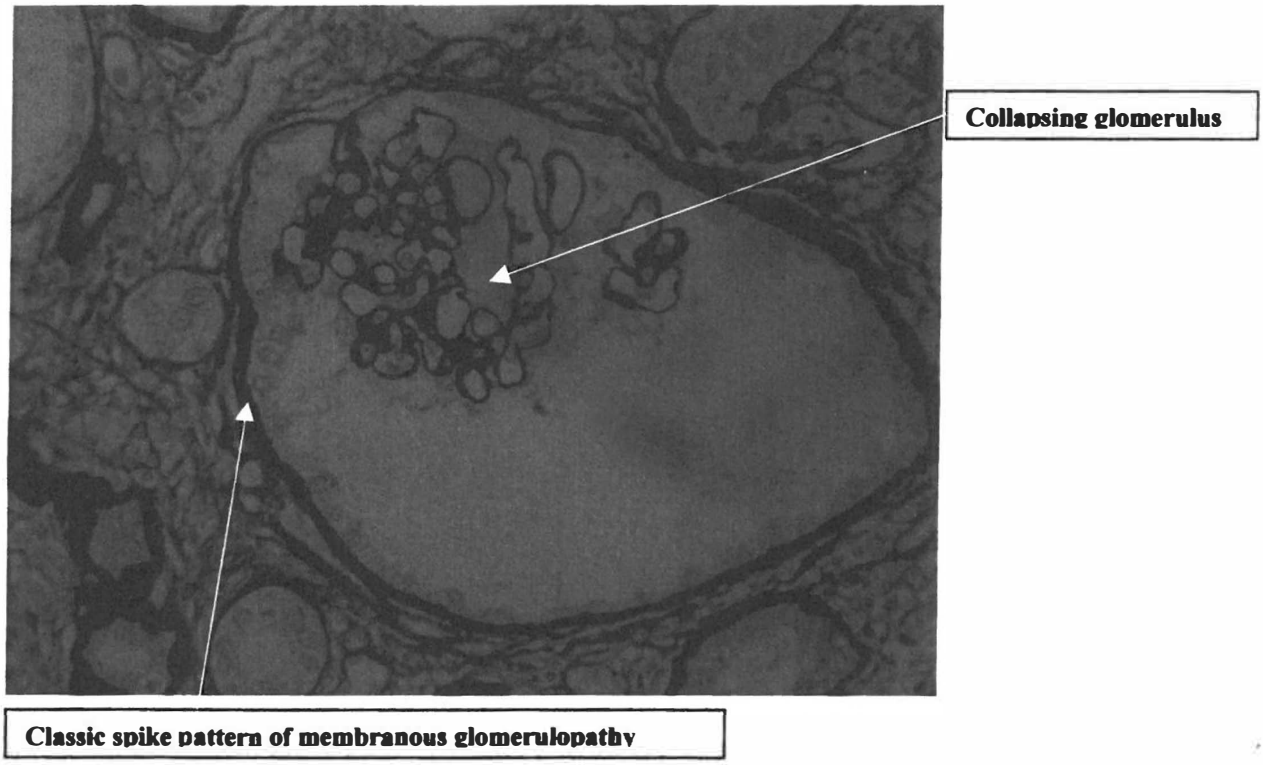


Figure 23 (b) . High power view of **Figure 23 (a)**

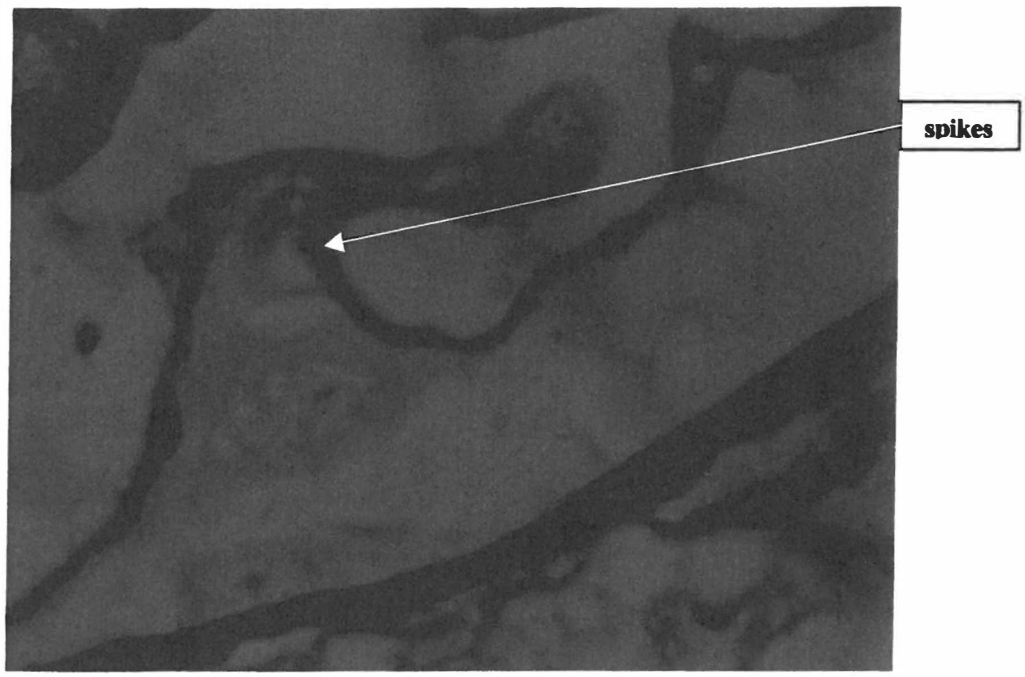


Figure 23(c). Electron microscopy of Figure 23 (a) showing subepithelial electron dense deposits

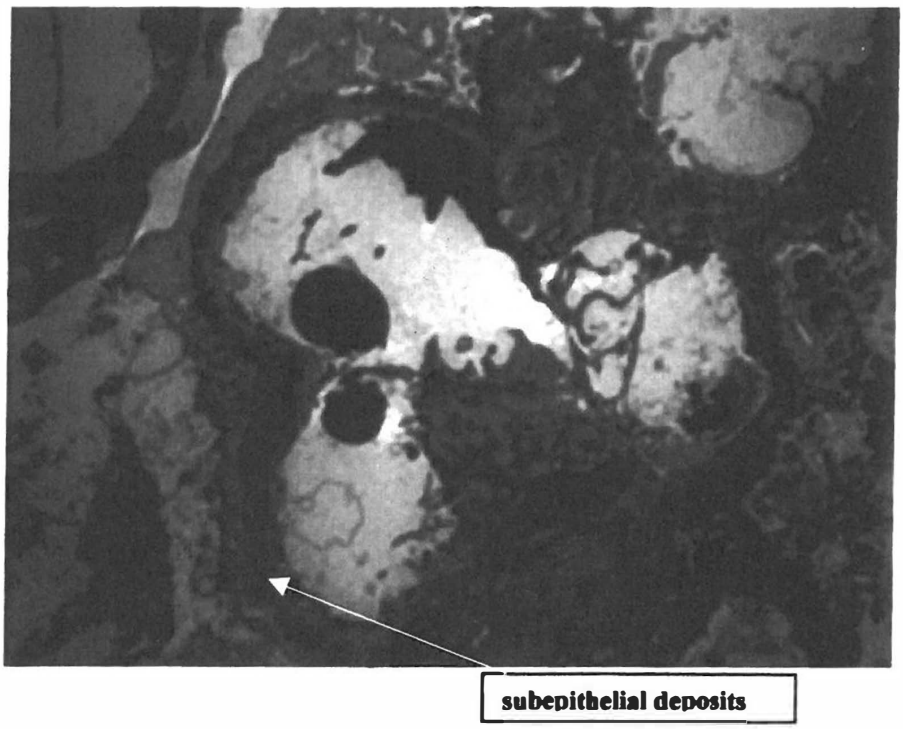


Table 10. Characteristics of patients with HIVAN

Patient	Age (yrs)	Race	Gender	CD4	Se creatinine (μmol/l)	Cr Clearance (ml/min) [Measured]	Cr Cl (ml/min/1.73m²) [Calculated]	Hepatitis Serology		Urine protein (gm/24hr)
								B	C	
1	29	B	F	3	77	88	99	(-)	NA	0.94
2	28	B	F	183	103	80	71	(-)	(-)	0.9
3	26	B	F	204	109	63	68	(-)	NA	0.06
4	34	B	F	246	62	95	123	(-)	(-)	0.30
5	40	B	M	-	93	78	100	(-)	(-)	0.23
6	43	B	M	114	70	91	137	(-)	(-)	0.04
7	27	B	F	379	68	104	116	(-)	(-)	0.17
8	21	B	F	137	61	130	138	(-)	(-)	0.07
9	26	B	M	586	90	117	114	(-)	NA	6.33
10	17	B	F	-	82	48	102	(-)	NA	2.9
11	43	B	M	-	213	39	38	(-)	(-)	11.64
12	25	B	M	392	72	138	148	(-)	(-)	1.14
13	24	B	F	335	112	49	66	(-)	NA	3.61
14	38	B	M	-	161	55	54	(-)	(-)	6.83
15	21	B	F	130	178	31	40	(-)	(-)	9.21
16	38	B	F	159	181	45	35	(+)	(-)	14.0
17	29	B	M	331	78	78	131	(+)	(-)	24.75
18	35	B	M	402	103	89	92	(-)	(-)	4.92
19	32	B	F	-	135	50	51	(-)	(-)	5.75
20	29	B	F	145	172	56	39	(-)	(-)	4.47
21	30	B	F	213	156	22	43	(-)	NA	1.35
22	26	B	F	-	155	31	45	(-)	NA	5.19
23	42	B	M	130	88	116	106	(-)	(-)	2.23
24	29	B	M	90	119	104	89	(-)	(-)	1.38
25	24	B	F	-	63	97	129	(-)	(-)	11.15

B=Black ; M=Male ; F=Female ; NA=not available

Table 11. Characteristics of patients with combined HIVAN and membranous nephropathy

Patient	Age (yrs)	Race	Gender	CD4	Se Cr (µmol/l)	Cr Cl (ml/min) [Measured]	Urine protein (gm/24hr)	Hepatitis Serology		ANF	WR
								B	C		
1	43	B	M	114	70	94	0.04	(-)	(-)	(-)	NR
2	38	B	F	159	181	45	14.0	(+)	(-)	(-)	NR
3	29	B	M	331	78	78	24.75	(+)	(-)	(-)	NR
4	24	B	F	-	63	97	11.15	(-)	(-)	(-)	NR

NR= Non-reactive

Table 12. Characteristics of patients with non-HIVAN (other renal diseases)

Patient	Age (yrs)	Race	Gender	CD4	Serum creatinine (µmol/l)	Cr Clearance (ml/min) [Measure/ Calculated]	Hepatitis serology		Urine protein (gm/24hr)	Renal biopsy
							B	C		
1	30	B	F	239	37	188/188	(-)	(-)	0.13	IN
2	29	B	M	641	198	48/45	(-)	(-)	12.98	MPGN
3	34	B	F	611	73	135/102	(-)	NA	0.47	IN
4	42	C	M	210	147	25/58	(-)	(-)	5.45	MPGN
5	40	B	F	39	85	80/83	(-)	(-)	0.48	IN

B=Black ; C=Colored ; IN=Interstitial Nephritis ; MPGN=Membranoproliferative glomerulonephritis ;
NA= not available

CHAPTER 5

DISCUSSION

The HIV-infected population is rapidly increasing in size. A human being is infected with HIV every 10 seconds on average (UNAIDS). In Africa, about 30 million people have HIV infection. Black patients have a relative risk of 51.1 for developing end-stage renal disease (ESRD) from AIDS or AIDS-defining diagnosis compared with white patients [Barisoni L., 2003]. In Africa, about 30 million people have HIV infection. However, the incidence of renal diseases associated with HIV infection in Africa is unknown. There is a paucity of published renal biopsy series from Africa. Pantanowitz *et al* (1999) reported renal biopsies of 21 Black HIV-infected patients from Johannesburg, which showed FSGS in 62%; mesangial proliferative, membranous and post-infectious glomerulonephritis 14% each and mesangiocapillary glomerulonephritis in 5%. Eighty one percent of the biopsies showed tubulo-interstitial nephritis. Acute tubular necrosis was found in 48% of biopsies and pyelonephritis in 14%. Renal tuberculosis was present in one case. Diallo *et al* (1997) studied 33 HIV-infected Black African patients with nephrotic syndrome in Abidjan over a 7 year period (1986-1993) and HIV associated nephropathy was found in 5(15%). Assounga *et al* (1996) performed a comparative study of HIV-positive versus HIV-negative patients from Congo, Brazzaville who presented with various renal problems. One hundred and seventeen patients were observed over one year period. A total of 43 (36.8%) were found to be HIV-positive; 32 of them underwent renal biopsy. Minimal change GN was found in 25%; focal and segmental hyalinosis in 18.8%; MPGN in 18.8%; proliferative endocapillary GN in 3%; complex GN in 12.5%; Interstitial nephritis in 6.3%; amyloidosis in 6.3% and nephrosclerosis in 3%.

The actual prevalence of HIVAN worldwide is uncertain. Available data were either based on renal biopsy or autopsy studies which gave variable results. Autopsy studies showed the

prevalence of HIVAN to be 1 to 12%, whereas a higher percentage was found in biopsy series. There are several renal biopsy series reported in the literature (Table 13).

Table 13. Renal biopsy series

Study	Country	Number (biopsies)	HIVAN (%)	Se Cr (µmol/l)	UP (gm/24hr)	Mean CD4	Remark
Bourgoignie (*KI*, 1990)	-	176	146(83%)	-	-	-	Literature review of biopsies reported before 1990
Casanova et al (*Am J Kidney Dis*, 1995)	Italy	26	0 (0%)	61-735	0.2 – 17.6	-	All white Italian
Connolly et al (*QJM*, 1995)	UK	19	11(58%)	137 - 1400	2.6 – 10.0	208 (<10 – 630)	8 out of 11 patients with HIVAN were Afro-Carribean
Assounga et al (*Saudi J Kidney Dis Transplant*, 1996)	Congo	32	6(18.8%)	-	-	-	All black African
Praditpornsilpa et al (*Am J Kidney Dis*, 1999)	Thailand	26	0 (0%)	53 –2917 (<133 in92%)	1.5 – 10.7	275 (11-560)	All Thai patients
Winston et al (*KI*, 1999)	USA	20	10(50%)	123 – 495 (<133 in 10%)	1.1 – 12.0	60 (0 – 200)	9 out of 10 patients with HIVAN were African American
Ahuja et al (*Am J Nephrol*, 1999)	USA	14	9(64%)	59 – 332 <133 in 44%)	All >1.5	187 (6 – 686)	All African American
Our study	South Africa	30	25(83%)	61-213 (<133 in 68%)	0.04 – 24.75	223 (3 –586)	All South African black patients

UP= urine protein ; HIVAN=HIV-associated nephropathy

Literature review by Bourgoignie (1990) of renal biopsies in 176 HIV-infected patients reported before 1990 revealed focal segmental glomerulosclerosis in 83%. However, racial groups were not mentioned in the review, although it is likely that majority was black. In the later series, HIVAN was also found to be the commonest form of renal involvement in HIV-infected black patients, ranging from 50% to 64%[Connolly *et al*, 1995; Winston *et al*,

1999; Ahuja *et al,* 1999]. In our study, 83% of patients had HIVAN which was higher than the later series. We screened both hospital in-patients as well as asymptomatic out-patients to cover different severity of disease. In terms of renal involvement, proteinuria is used as a marker. In our study, patients with variable degrees of proteinuria, ranging from microalbuminuria to nephrotic range proteinuria, were screened and recruited. Therefore, this study covered both extremes of proteinuric patients from in- and out-patient groups. We screened a total of 615 patients; 15(2.4%) were in-patients and 600 (97.6%) were out-patients; 38 (6%) had overt proteinuria and 7(7.8% of 90 patients tested) had persistent microalbuminuria.

HIV infection can affect the kidney and give rise to various forms of nephropathy. Among the various histological types we found in our study patients, it is interesting to note that 2 different histological patterns i.e HIVAN and membranous nephropathy occurred simultaneously in 4 patients. Two of them were Hepatitis BsAg positive which could explain the membranous nephropathy. However, the other 2 had no explanation for this type of nephropathy. HbsAg was negative both by the rapid and ELISA tests done at the Virology laboratory. PCR for hepatitis B was not done. Although, in theory, PCR could be positive while HbsAg is negative, this is unlikely in a patient with a chronic infection. Therefore, both histological patterns may be directly related to HIV infection. The occurrence of two different histological patterns in the same patient simultaneously has never been reported in the literature. Characteristics of these patients with combined HIVAN and membranous nephropathy are shown in the Table 11. The heaviest degree of proteinuria was found in 3 (75%) of these patients.The presence of dual pathology, therefore, may be responsible for the massive proteinuria in these patients.

There are various subtypes of HIV-1; subtype B being the commonest in the United States, North America and Europe, whereas subtype C is accountable for the vast majority of infections in Africa [Quinn *et al.*, 1998]. Casanova *et al* (1995) did a biopsy study in white Italian HIV-positive patients, however HIVAN was not demonstrated. Therefore, the HIV-1 subtype may be one of the determining factors for the pattern of renal disease among the patients. However, the study done by Praditpornsilpa *et al* (1999) in Thailand, where subtype C is predominant as in sub-Saharan Africa, did not demonstrate a single case of HIVAN among 26 HIV-positive patients presenting with varying degrees of proteinuria. It is, therefore, possible that both race or genetic factors and HIV-1 subtype may play a role in the development of HIVAN.

The pathogenesis of HIVAN is unclear which makes recommendation of specific therapy difficult. Relentless progression of renal dysfunction in patients with HIVAN and lack of specific therapy for the disease leads to poor outcome in such patients. Corticosteroids, ACE inhibitors and anti-retroviral therapy have been used in small case series and in anecdotal reports; however, prospective randomised controlled studies are lacking. Some uncontrolled studies were done to determine the use of corticosteroids in HIVAN, but its efficacy and safety remain uncertain. Anti-retroviral therapy (ARV) has been recommended to improve renal functional abnormalities and outcome in patients with HIV infection [Ifudu *et al.*, 1995; Wali *et al.*, 1998; Dellow *et al.*, 1999; Winston *et al.*, 2001]. However, it has only recently been launched in government health services in South Africa and is not widely available to the majority of HIV-infected patients. Therefore, it is not current practice to use ARV in HIV associated renal disease.

Prolonged renal survival with the use of ACE inhibitors has been well established in various proteinuric nephropathies [Ruggenenti *et al.*, 1999; Taal *et al.*, 2001; Chan *et al.*, 2000;

Parving *et al.*, 2001]. Based on these findings, ACEI becomes an option in managing patients with HIVAN. Wei *et al* (2003) recently showed the sustainable long-term renoprotective effect of ACEI in patients with HIVAN. Renal survival was significantly prolonged with ACE inhibition, independent of nephrotic status, anti-retroviral exposure, low CD4 count, or presence of moderate to severe chronic interstitial changes. The therapeutic effect of ACEI may be multifactorial [Kimmel *et al.*, 1996]. Inhibition of ACE might affect HIVAN through renal haemodynamic changes or through the action at the molecular level, by down-regulating the expression of growth factors such as transforming growth factor-β (TGF-β), which plays a role in the pathogenesis of experimental glomerulosclerosis [Border *et al.*, 1992 & 1994; Sharma *et al.*, 1993]. It has antiproliferative effects, resulting in increased net extracellular matrix synthesis and decreased degradation. Elevated plasma and tissue TGF-β levels have been reported in patients with AIDS [Kekow *et al.*, 1990; Allen *et al.*, 1991]. TGF-β increases the expression of the HIV-1 LTR promoter in vitro in human mesangial cells [Shukla *et al.*, 1993]. Renal cellular synthesis of TGF-β is increased by angiotensin II (AT II) [Hahn *et al.*, 1991; Stouffer *et al.*, 1992; Koibuchi *et al.*, 1993]. AT II has also been shown to stimulate another inflammatory modulator, NF-κB [Guijarro *et al.*, 2001]. Therefore, it is possible that, by inhibiting ACE, ACEI decreases AT II, thereby reducing renal tissue expression of TGF-β and NF-κB, resulting in delayed progression of the renal disease. Human immunodeficiency virus proteases may have activity in the rennin-angiotensin cascade and serum ACE levels have been found to be elevated in HIV-infected patients [Ouellete *et al.*, 1992]. ACEIs are protease inhibitors and this inhibition might have a salutary effect at the tissue level [Kimmel *et al.*, 1996].

The anti-proteinuric effect of ACEI was clearly shown in patients with significant proteinuria (>1gm/24hour) in our study. However, this effect was not obvious in the group

with lower degrees of proteinuria (<1gm/24hour), although the amount of proteinuria was found to be stable at the end of the study. It is likely that the level of proteinuria was too small to be observed as a significant reduction. The anti-proteinuric effect of ACEI in this group, therefore, may be considered as a stabilization rather than reduction of proteinuria, which was evident in this particular group. Improvement in serum creatinine and Cr Cl in this group, although statistically non-significant, may be viewed as a renal protective effect of ACEI. These results did not reach significant level probably due to the small number of study patients. A longer duration of follow-up on larger group of patients is desirable to clarify these issues. The majority of patients in this group either defaulted or died from non-renal causes, which limits our study. Regular attendence to the health services is a major problem in our patient population due to various socio-economic reasons. Increased mortality among the study patients from HIV-related diseases, yet with good renal function is the another major limitation to our study. In patients with significant proteinuria of >1gm/24hour, with ACEI therapy, Cr Cl was better preserved and the degree of anti-proteinuric effect correlates with the duration of follow-up, suggesting that long term use of the drug may result in improved efficacy. The initial Cr Cl was found to be significantly lower in proteinuric patients compared with patients with microalbuminuria, showing inverse relationship of renal function and degree of proteinuria, supporting the well known nephrotoxic effect of proteinuria.

A salient feature of this study was the inclusion of patients with microalbuminuria. Those patients with *persistent* microalbuminuira were selected to represent early, subclinical renal involvement in otherwise asymptomatic HIV-infected patients. Microalbuminuria has been assessed by various investigators to diagnose subclinical renal involvement in systemic diseases. Valente *et al* (1998) performed renal biopsies in patients with systemic lupus

erythematosus who had microalbuminuria but without clinical signs of renal involvement, to evaluate subclinical lupus nephropathy. Fifteen patients (50%) had mesangial glomerulonephritis (MGN) type IIb, 12 (40%) had MGN type IIa and 3 (10%) patients showed no changes on light microscopy or on immunofluorescence (type 1). Similarly, microalbuminuric diabetic patients were biopsied to detect the presence of diabetic nephropathy. Thirty four type 2 diabetic patients with microalbuminuria were biopsied by Fioretto *et al* (1996); 10 (29.4%) had typical diabetic nephropathy, 10 (29.4%) showed normal or near normal structure, 14 (41.2%) revealed atypical patterns of injury, with absent or only mild diabetic glomerular changes. Kanauchi *et al* (2001) also performed renal biopsies in 23 type 2 diabetic patients with microalbuminuria; 13 (57%) had typical diabetic glomerulosclerosis and 10 (43%) revealed atherosclerotic nephropathy without evidence of diabetic glomerulopathy. Studies were also done to detect an association between microalbuminuria and hypertension [Parving *et al.*, 1974]. According to these findings, microalbuminuria appears to be an early indicator of renal disease in patients with systemic diseases. Therefore, microalbuminuria detected in HIV-positive patients may also indicate early (subclinical) HIVAN or may be a marker of non-HIV nephropathy.

Luke *et al* (1992) measured microalbuminuria in 72 HIV-seropositive ambulatory patients. There were 14 patients (19.4%) who had increased urinary microalbumin levels; 7 of these patients had nephrotic range proteinuria, similar to those values found in diabetic nephrotic syndrome. Two other studies showed the prevalence of microalbuminuria in HIV-infected out-patients without clinical evidence of renal disease as 29.8% and 25% respectively [Kimmel *et al.*, 1993; Kabanda *et al.*, 1996]. However, renal biopsy was not performed in these patients to determine the pattern of renal pathology. To our knowledge, there are no renal biopsy studies done in HIV-infected patients with *microalbuminuria* to date. It was

considered that choosing those patients with microalbuminuria (which was persistent) would improve the detection of patients whose kidneys were silently affected and would rule out those with false positivity. In our study, 7 patients with microalbuminuria underwent renal biopsy and 6 (86%) patients showed HIVAN and 1(14%) had interstitial nephritis. Therefore, all patients were found to have histological abnormalities with almost all being discovered as having HIVAN, which is probably more than mere chance, although the small number may not allow statistically meaningful conclusions. Interestingly, the microalbuminuric group constituted 24% of all patients with histological evidence of HIVAN. However, these patients had good renal function [mean initial Cr Cl of 139 ml/min] and had low initial CD4 count [mean of 216]. Of the patients with HIVAN, the majority had low CD4 (<250 in 12 out of 18 patients whose CD4 counts were available), in keeping with other reports [Winston *et al.*, 1998 & 1999]. It is, therefore, possible that HIVAN occurs in patients with low CD4 counts and can present initially with microalbuminuria which has to be persistent. The beneficial effect of ACEI in patients with microalbuminuria was unclear in our study; due to small numbers of patients, early mortality and non-compliance. Larger number of patients with a longer follow-up period is required to address this issue.

When comparing the characteristics of patients who were evaluated for proteinuria, there were no significant differences in age, sex, race, and mean blood pressure among the 3 different groups (Table 2). However, one unanticipated finding in our study was that the mean CD4 count was the lowest in normoalbuminuric patients compared to micro- and macro-proteinuric groups, although both latter groups also had low counts. The reason for this is unclear. It may be a reflection of advanced HIV infection generally in all patients seeking medical attention. However, subclinical renal involvement in the apparently normoalbuminuric group is not excluded as the majority were not tested for

microalbuminuria. As persistent microalbuminuria was found in 7.8% amongst those whom we tested, it is likely that more patients with microalbuminuria would have been detected, had they all been tested. Then, subsequent renal biopsy, if done, would have shown the renal abnormalities, as was the case in our study.

CHAPTER 6

CONCLUSION

The number of people living with HIV has been rising in most regions and South Africa still remains the worst affected region in the world. As the HIV-infected population grows, so will be the number of patients with varying degrees of renal involvement who ultimately will impose a threat on currently overburdened renal services in the region. Preventive measures still need to be re-enforced in an attempt to reduce the number of new cases. Care of the already-infected patients should be optimised, including provision of anti-retroviral therapy to a larger scale. Patients should be regularly screened for the presence of proteinuria. Those with overt proteinuria and renal impairment, should have histological diagnoses established at an early stage of the disease. ACEI therapy needs to be initiated as early as possible in patients with HIVAN and all proteinuric nephropathies in HIV-infected patients, which is the only promising therapy widely available to our patient population currently. HIVAN should be considered as an AIDS defining illness and ARV initiated. Microalbuminuria is a manifestation of HIVAN in our study patients. Most patients with HIVAN had low CD4 count (<250/mm^3). Screening for microalbuminuria may be beneficial in HIV-infected patients with low CD4 count. Renal biopsy may be a consideration in those with persistent microalbuminuria, in order to diagnose HIVAN at an early stage and to achieve the maximal benefits of ACE inhibition. Further prospective randomised controlled trials with ACEI and ARV are needed.

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