RELEVANCE OF MICROALBUMINURIA IN SCREENING FOR HIV-ASSOCIATED NEPHROPATHY

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Master of Medicine in the branch

of

Paediatrics

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DECLARATION

I, Bhadrish Jayantkumar Mistry declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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27 day of February, 2009

DEDICATION

This work is dedicated to my parent's Jayantkumar Rama and Nirmela Mistry for teaching me the values of perseverance and commitment.

To my wife, Kalpa and son's Khushil and Prem for their love, patience and inspiration.

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ABSTRACT

Introduction

HIV-Associated Nephropathy (HIVAN) is the commonest cause of proteinuria, especially in black HIV seropositive children. This chronic nephropathy is a late complication of untreated HIV that requires earlier intervention to prevent progression of renal disease. Microalbuminuria is an early marker of the presence of subclinical renal disease in systemic diseases such as diabetes mellitus and hypertension. This study assessed the prevalence and clinical significance of a single screening test for microalbuminuria in a cohort of HIV seropositive children without any symptoms of renal disease at Chris Hani Baragwanath hospital situated in Johannesburg, South Africa.

Methods

A prospective study was undertaken at Chris Hani Baragwanath hospital (a major tertiary facility that serves the people of Soweto and the surrounding areas of southern Gauteng). HIV seropositive and seronegative patients from both an inpatient and outpatient ambulatory setting were screened for qualitative proteinuria and microalbuminuria. Those on antiretroviral therapy, anti tuberculosis treatment, known chronic kidney disease, hypertension, fever, acute illness and urinary tract infection were excluded from the study.

Results

180 patients were enrolled into the study, of which 110 were HIV positive and 70 HIV negative. Majority of the patients were black (98%) with 100 (56%) males and 80 (44%) females. Microalbuminuria was present in 27(25%) of HIV positive patients and 1 (1%) HIV negative patient, p=0.00003. The mean age at presentation of microalbuminuric HIV positive patients was 6 ± 3.2 years. With normal renal function and no proteinuria; microalbuminuria was present in 21 (19%) patients, p=0.03. Microalbuminuric patients were moderately immunosuppressed (mean CD4 % of 16.8 ± 8%, mean viral load 8 ± 18 x 10^5 RNA copies/ml) and had WHO clinical stage 2 and 3 disease. Absolute CD4 counts appear to correlate better with microalbuminuria than CD4 percentage as the mean CD4 absolute count in HIV positive patients with microalbuminuria (493 ± 330 x 10^6/l) was significantly lower than those without microalbuminuria (780 ± 702 x 10^6/l), p=0.03.

Conclusion

Microalbuminuria screening of HIV positive patients is a more sensitive screening test compared to standard urine dipsticks as it is present in patients with normal renal function who have no proteinuria. This may allow for early identification of subclinical renal disease in patients with some evidence of immunosuppression; thus possibly preventing the deterioration of renal function and severity of HIV disease with early initiation of antiretroviral therapy.

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LIST OF ABBREVIATIONS

AIDS – Acquired Immunodeficiency Syndrome

CD4 – Cluster of differentiation 4

CDC – Centre for Disease Control and Prevention

ESRD – End stage renal disease

GFR – Glomerular filtration rate

FGF – Fibroblast growth factor

FSGS – Focal segmental glomerulosclerosis

HIV – Human immunodeficiency virus

HIVAN – Human immunodeficiency virus -associated nephropathy

HUS – Hemolytic uremic syndrome

IL – Interleukin

INF – Interferon

LIP – Lymphoid interstitial pneumonia

LRTI – Lower respiratory infection

MA – Microalbuminuria

PDGF – Platelet derived growth factor

RANTES - Regulated on Activation, Normal T Expressed and Secreted

TB – Tuberculosis

TGF – Transforming growth factor

TNF – Tumour necrosis factor

TTP – Thrombotic thrombocytopenic purpura

URTI – Upper respiratory infection

WHO – World Health Organization

CHAPTER 1

INTRODUCTION

1.1 HIV-associated nephropathy (HIVAN)

Since the first recorded case of Acquired Immune Deficiency Syndrome (AIDS) and the identification of Human Immunodeficiency Virus Type 1 (HIV) as the causative agent of AIDS, the HIV/AIDS epidemic has spread at an alarming rate throughout the world particularly in sub-Saharan Africa. UNAIDS (2007) estimates about 2.1 million children worldwide are infected with HIV. Although sub-Saharan Africa is home to just over 10% of the world's population, nearly 90% of all children with HIV live in this region.[1]

South Africa alone has almost a third of all new HIV infections and AIDS related deaths globally in 2007. According to the Actuarial Society of South Africa the estimated HIV prevalence for children less than 18 years has almost doubled from 1.2% in 2000 to 2.1% in 2006; with children less than 5 years of age being most affected.[2]

Although HIV is a multisystem disease, the kidneys are commonly affected. The spectrum of kidney disease in HIV is extensive and ranges from acute to chronic. HIV associated nephropathy (HIVAN) is a chronic a disorder which was first described in adults and children with AIDS in New York and Miami in 1984.[3] It is characterized by proteinuria and haematuria and the rapid development of renal insufficiency leading to end stage renal disease (ESRD).

1.2 Prevalence

The worldwide prevalence of childhood HIVAN is approximately 10-15%, especially in African American children.[4,5] However, the true prevalence in African children is unknown due to a lack of surveillance and reporting of kidney disease in HIV positive children.

1.3 Pathogenesis

The pathogenesis of renal disease in HIV infection is not completely understood. It is a complex inter-relationship between the HIV-1 which directly infects renal epithelial cells in a genetically susceptible host causing podocyte dysregulation and proliferation as well as mesangial hyperplasia. Various cytokines (TNF α, IL-2, INF-γ), growth factors (PDGF, TGF-β, FGF-2) and chemokines (IL-8, RANTES, monocyte chemo-attractant protein I) that are produced by HIV infected cells have been shown to play a role in the pathogenesis of HIVAN.[6,7,8]

1.4 Clinical presentation

HIVAN encompasses distinct syndromes; classic focal segmental glomerulosclerosis which is collapsing in nature (FSGS), immune complex mediated, mesangial hyperplasia and thrombotic thrombocytopenic purpura/ hemolytic uremic syndrome (TTP/HUS).[7]

Proteinuria seems to be the earliest and most consistent finding for the diagnosis of HIVAN.[9,10] The degree of proteinuria is variable, from minimal to nephrotic range.

The classical picture of nephrotic syndrome with peripheral oedema and hypercholesterolaemia is often absent but may be seen.[7] Whether such clinical findings represents distinctive characteristics of the nephropathy or are simply indicative of overall health and nutritional status of the patients with advanced HIV disease is debatable.

In immune complex disease hypertension and haematuria are common. These patients have an indolent course and are less likely to progress to ESRD. Mesangial hyperplasia may reflect an early stage of FSGS, however this there insufficient data to support this hypothesis.[11] Patients with mesangial hyperplasia have proteinuria, are older and have an excellent prognosis as the rate of progression to renal failure is much slower. Patients with TTP/ HUS disease usually present with microangiopathic haemolytic anemia and mild to moderate renal insufficiency together with other features such as fever, thrombocytopenia and neurological changes.[7]

1.5 HIVAN in children and adults

In earlier reports of renal disease in HIV infected individuals, some noticeable differences existed between HIVAN in adults and children. In adults, HIVAN tends to progress rapidly to ESRD and is associated with a higher mortality rate. Whereas in children, progression to ESRD is slow, variable and highly dependent on the histological lesion.[12]

Children with focal glomerulosclerosis or segmental necrotizing glomerulonephritis tend to develop severe renal failure within 1 year of diagnosis and have a high mortality rate, usually from cause's unrelated to renal insufficiency.[12] In adults and children, outcome is dictated by progression of AIDS and is independent of the primary renal disease.[6,13]

1.6 Diagnostic evaluation of HIV-associated nephropathy

HIVAN may be the first manifestation of infection with HIV before the development of AIDS, although it predominantly occurs as a late stage complication.[14] Most patients have advanced renal failure or AIDS defining illnesses at the time of diagnosis. The length of time from detection of nephropathy to severe renal failure averaged 9 months with a range from 1 to 27 months.[12]

The kidneys are often large and echogenic on renal imaging studies. The predictive value of renal ultrasound to prove or exclude the diagnosis of HIVAN has not been studied. The only reliable test to establish the presence of HIVAN is a renal biopsy. The more commoner glomerular abnormality is focal segmental glomerulosclerosis. However, a spectrum of lesions may be present, and virtually all types of glomerular lesions have been described.[15,16] According to two South African studies, FSGS and immune mediated complex disease are common in children and adults.[17,18]

1.6.1 Microalbuminuria

The normal rate of protein excretion in children is <4mg/ m^2/hour or <100mg/m^2/day throughout childhood in both boys and girls.[19] Albumin excretion in spot urine in the range of 20 to 200mg/l is referred to as microalbuminuria.[20,21] At these levels, the standard dipsticks do not detect albumin in the urine and thus specific measurement of albumin using high albumin-sensitive techniques are required.

Microalbuminuria, a type of glomerular proteinuria, is an early marker for the development of nephropathy and generalized endothelial dysfunction in diabetes.[22] Similarly, in non diabetics, increased urinary albumin excretion may also indicate a renal glomerular disease.[23,24]

Microalbuminuria may precede proteinuria and may be the only manifestation of HIV disease before the AIDS complex manifests.[12] The prevalence of microalbuminuria in HIV seropositive adults varies. It has been identified in 19.4% of HIV-infected adults and is inversely correlated with CD4 T-cell counts; however no prospective information is available regarding the progression to clinically significant renal disease in these patients.[25] Han TM et al reported microalbuminuria in 36% of HIV-infected adults.[9] In the Fat Redistribution and Metabolic Change in HIV Infection Study; microalbuminuria was present in 11% of HIV-infected adults, but only in 2% of controls.[26]

1.7 Aims of the study

In view of the paucity of data on the prevalence and clinical significance of microalbuminuria in HIV-seropositive children; a study was undertaken to assess microalbuminuria in relation to the clinical presentation of HIV/AIDS. Microalbuminuria is an independent risk factor for the development of chronic kidney disease and loss of glomerular filtration.[22] Adult studies have shown that microalbuminuria is an early manifestation of HIVAN.[9,12] Thus, the screening for urinary microalbumin may allow for early identification of renal involvement in patients with HIV/AIDS infection.

CHAPTER 2

MATERIALS AND METHODS

2.1 Ethics clearance

Approval was obtained from the Ethics Committee of the University of Witwatersrand.

Clearance no: M050808

2.2 Study sample

A prospective, single centre trial was undertaken at Chris Hani Baragwanath Hospital (the major tertiary referral facility that serves the people of Soweto and surrounding areas of southern Gauteng), Department of Paediatrics, South Africa from March 2006 to December 2007. Patients were recruited aged 6 months to 16 years that were attending the HIV outpatient clinic (Harriet Shezi) and in patients from our general paediatric wards.

The HIV status of patients was determined by enzyme linked immuno-absorbent assay (ELISA) or polymerase chain reaction (PCR). The patients were divided into HIV infected or HIV uninfected groups.

All HIV positive patients were on Trimethoprim-sulphamethoxazole prophylaxis for the prevention of Pneumocystis jiroveci. The study sample comprised 110 HIV seropositive and 70 HIV seronegative patients.

Exclusion criteria were as follows:

- HIV positive patients on antiretroviral therapy
- HIV positive patients on anti-tuberculosis treatment
- Known chronic kidney disease (NKF KDOQI Clinical Practice guidelines for CKD in Children and Adolescents[27])
- Hypertension (blood pressure tables for adolescents and children, infants [28])
- Fever
- Acute illness
- Urinary tract infection.

Standard urine dipstick testing was performed using MAKROmed strips (Makromed Manufacturing, Judith's Paarl, Johannesburg, South Africa) in all patients. Patients with features of urinary tract infections and glycosuria were excluded. A urine dipstick reading ≤ 20 mg/dl (negative and trace) of protein was considered negative proteinuria and > 20mg/dl (≥ 1+) was labelled proteinuria.[27]

The presence of microalbuminuria was assessed by Micral-Test strips (Roche Diagnostics, Laval, Québec, Canada) as per the manufacturer's instructions. A single morning urine specimen in all patients was collected.

This is a simple, convenient and a reliable estimate of quantitative microalbumin excretion and it correlates well with the 24 hour urine collection.[29] Urine excretion of 20-200mg/l was defined as microalbuminuria.[30] Thus patients were categorized into negative (<20mg/l) and positive (≥ 20mg/l) microalbuminuria. Confounding factors such as fever, exercise, acute illness, urinary tract infection, hypertension and diabetes mellitus and anti-tuberculosis treatment were excluded. Glomerular filtration (GFR) was calculated using the Schwartz formula and expressed as milliliters/minute/1.73 metre squared (ml/min/1.73m^2)[31].

2.3 Data collection

Informed consent was obtained from the parent and assent from patients over 7 years of age. Demographics (age, sex, and race), nutritional status, comorbid conditions (diabetes mellitus, hypertension, and tuberculosis), current clinical presentation and laboratory measurements (full blood count, serum creatinine, serum total protein, serum albumin, CD4 count, and viral load) were extracted from patients' medical records. All data was captured and edited on Microsoft® Access 2003.

2.4 Statistical analysis

Statistical analysis was performed using Statistica 7.1 (StatSoft, Inc. 1984-2006, USA). Descriptive statistics using means, medians, standard deviation and confidence intervals were performed on all variables where appropriate.

Patients with and without microalbuminuria in both the HIV positive and negative groups were compared using the student's t test (dependent or independent samples) for continuous variables, and the Fisher's Exact or Chi-square tests were used for categorical data. P values < 0.05 were regarded as significant.

CHAPTER 3

RESULTS

A total of 180 patients were enrolled into the study; of which 110 were HIV positive and 70 HIV negative from both an inpatient and an outpatient ambulatory setting.

3.1 Age and racial distribution

There were 100 (56%) males and 80 (44%) females, with a ratio of 1.3:1. The majority of patients were black 177 (98%), reflecting the population of the catchment area, while 3 (2%) were of mixed races. The overall mean age at presentation was 4 years; ranging from 6 months to 16.5 years, see table 1. Majority of the HIV seropositive patients were older than 5 years with a mean of 5.8 ± 3.2 years at presentation but the HIV seronegative patients were younger than 5 years of age with a mean of 2 ± 2.1 years, p=0.0002.

Table 1 Age at presentation

Age (months)	HIV positive n=110 No. (%)	HIV negative n=70 No. (%)	P value
6 to 12	6 (5.5)	22 (31)	0.73
13 to 60	36 (33)	42 (60)	0.0006
60 to 120	59 (54)	5 (7)	0.007
> 120	8 (7)	1 (1)	0.4

3.2 Nutritional status

Figure 1 summarises the nutritional status (CDC 2000 growth charts [32]) of HIV positive and HIV negative patients. 26 (24%) HIV positive patients and 1(1.4%) HIV negative patient with recumbent length or stature greater than 121cm were not defined according to the CDC weight for stature growth charts. The mean nutritional assessment of the entire population was optimal, even though significant differences were noted in length or height and weight for length or height in both groups. However, this is due to patients being either adequately nourished or extremely malnourished, but generally the HIV negative patients were more malnourished as expected for length or height (p=0.005) and weight for length or height (p=0.004).

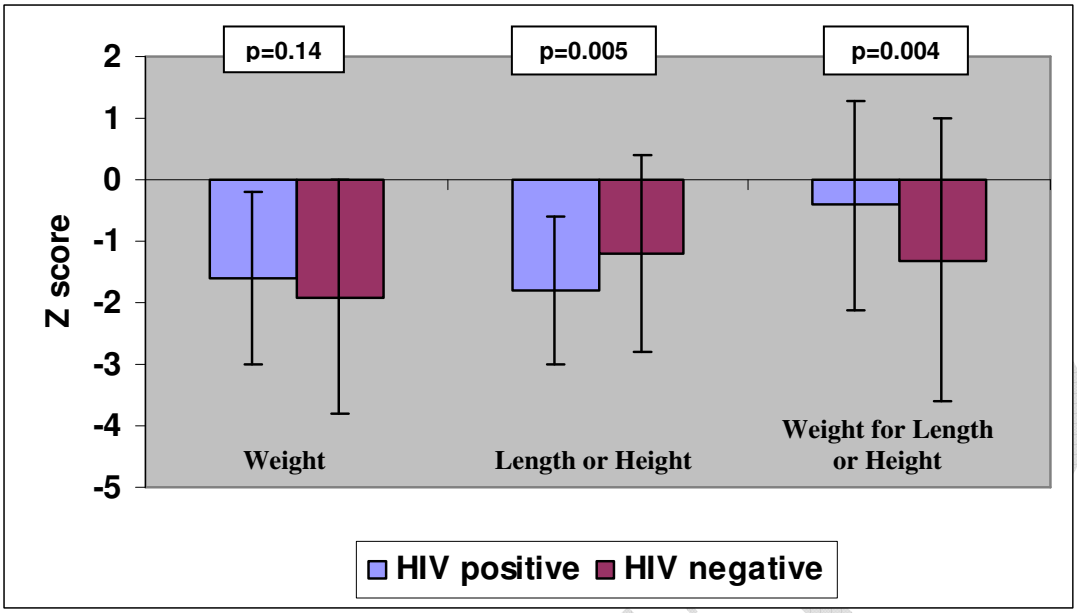

Figure 1 Nutritional status of HIV positive and HIV negative patients

3.3 Clinical features

The primary clinical features at the time of screening for both the HIV positive and negative patients are summarised in table 2. Lower respiratory tract infection was the commonest presentation in both groups.

Table 2 Clinical characteristics at presentation

	HIV positive	HIV negative
	n=110 No. (%)	n=70 No. (%)
Upper respiratory tract infection	18 (16.4)	5 (7.1)
Lower respiratory tract infection	28 (25.5)	20 (28.6)
Cardiovascular disease	-	3 (4.3)
TB pleural effusion	1 (0.9)	-
Gastroenteritis	5 (4.5)	21 (30)
Central nervous system disease	2 (1.8)	10 (17.3)
Other	10 (9.1)	18 (28.7)

3.4 Microalbuminuria

Microalbuminuria was present in 28 (15%) patients. 3 patients with indeterminate microalbuminuria due to tuberculosis treatment were excluded. An overview of patients tested for microalbuminuria is summarized in figure 2. Of the 28 patients with microalbuminuria, 27 (25%) were HIV positive and 1 (1%) HIV negative, p=0.00003. Thus indicating probable early renal disease in the HIV positive group.

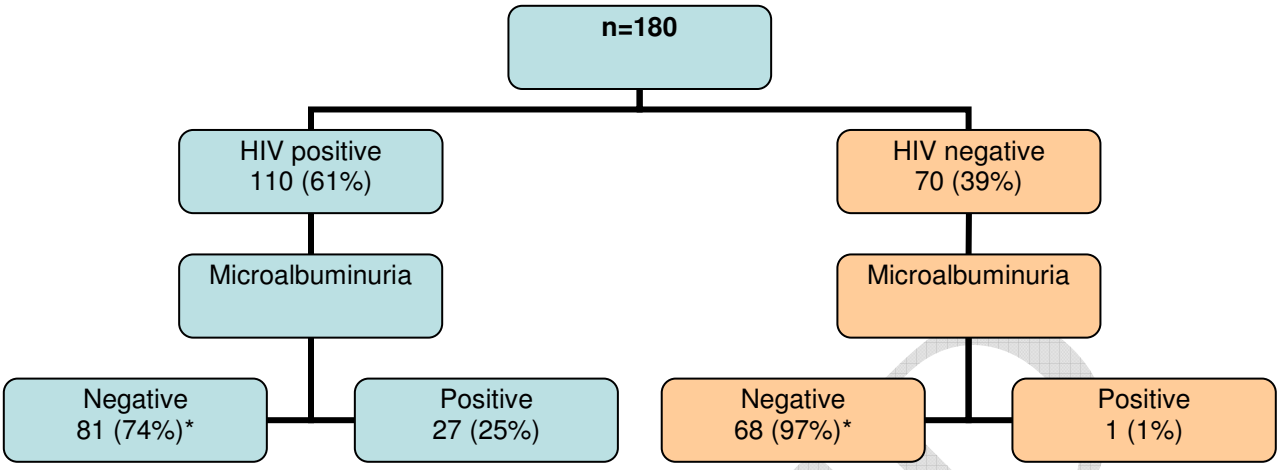

Figure 2 Overview of patients tested for microalbuminuria

* 3 patients (2 HIV positive and 1 HIV negative) with indeterminate microalbuminuria due to tuberculosis treatment were excluded

3.4.1 HIV positive group (n=27)

3.4.1.1 Age at presentation

The mean age at presentation of HIV positive patients with microalbuminuria was 6 ± 3.2 years (1-11.3 years) and those patients without microalbuminuria was 6 ± 3.3 years (0.6-16.6 years), p=0.6.

3.4.1.2 Nutritional status

The nutritional status of patients with and without microalbuminuria was optimal with no statistical differences, see figure 3.

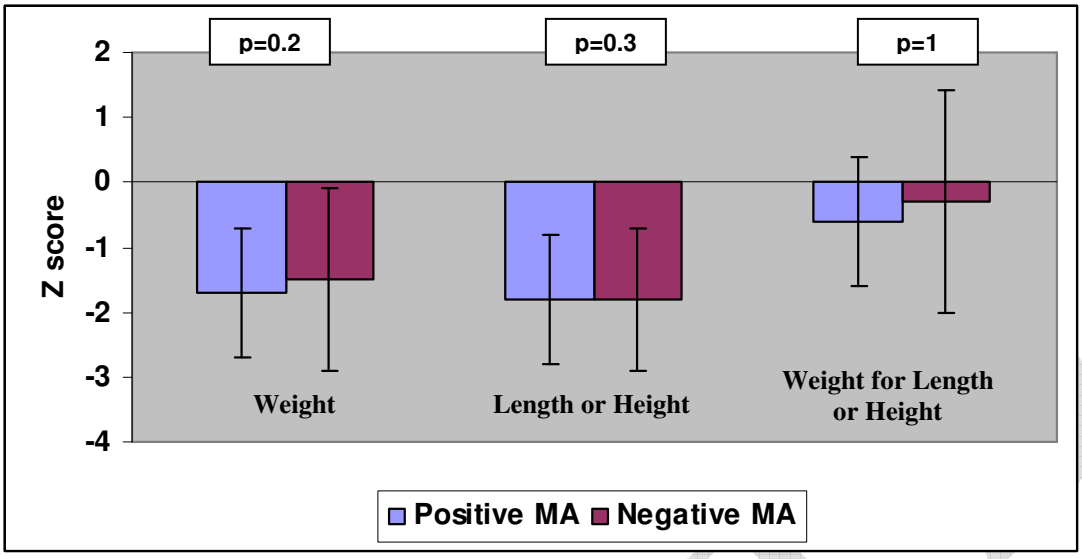

Figure 3 Nutritional status of patients positive and negative microalbuminuria

3.4.1.3 Clinical presentation

Of the 27 patients that presented with microalbuminuria, 19 (70%) were symptomatic and 9 (30%) were asymptomatic. The clinical characteristics of HIV positive patients with microalbuminuria were categorised according to the revised WHO clinical staging of HIV/AIDS [33], and are represented in table 3.

Table 3 Clinical characteristics of HIV positive patients tested for microalbuminuria

	WHO Staging							
	1		2		3		4	
WHO Staging Condition	**No. (%)**		**No. (%)**		**No. (%)**		**No. (%)**	
Asymptomatic		1 (4)						
Persistent generalized lymphadenopathy	13 (16)	2 (7)						
Hepatosplenomegaly			5 (6)	6 (22)				
Papular pruritic eruptions				1 (4)				
Parotidmegaly			3 (4)	1 (4)				
Herpes zoster			2 (2)					
Extensive molluscum			1 (1)					
Recurrent or chronic URTI			6 (7)	1 (4)				
Moderate unexplained malnutrition					10 (12)			
Pulmonary TB					20 (25)	8 (30)		
Severe recurrent LRTI					4 (5)			
Chronic HIV-associated lung disease including bronchiectasis						1 (4)		
Lymphoid interstitial pneumonia					10 (12)	3 (11)		
Severe unexplained malnutrition							7 (9)	1 (4)
Extrapulmonary TB							2 (2)	1 (4)
HIV encephalopathy							4 (5)	2 (7)
Disseminated non-tuberculous mycobacterium infection								2 (7)

Some patients have >1 clinical feature from the same stage, Shaded areas denotes negative microalbuminuria and unshaded areas denotes positive microalbuminuria

Tuberculosis and lymphoid interstitial pneumonia (LIP) was common among HIV positive patients. However, 40% of patients with LIP had associated tuberculosis. This association was present only in patients without microalbuminuria. HIV positive patients with microalbuminuria had predominantly stage 2 (30%) and 3 (37%) disease clinically, see figure 4.

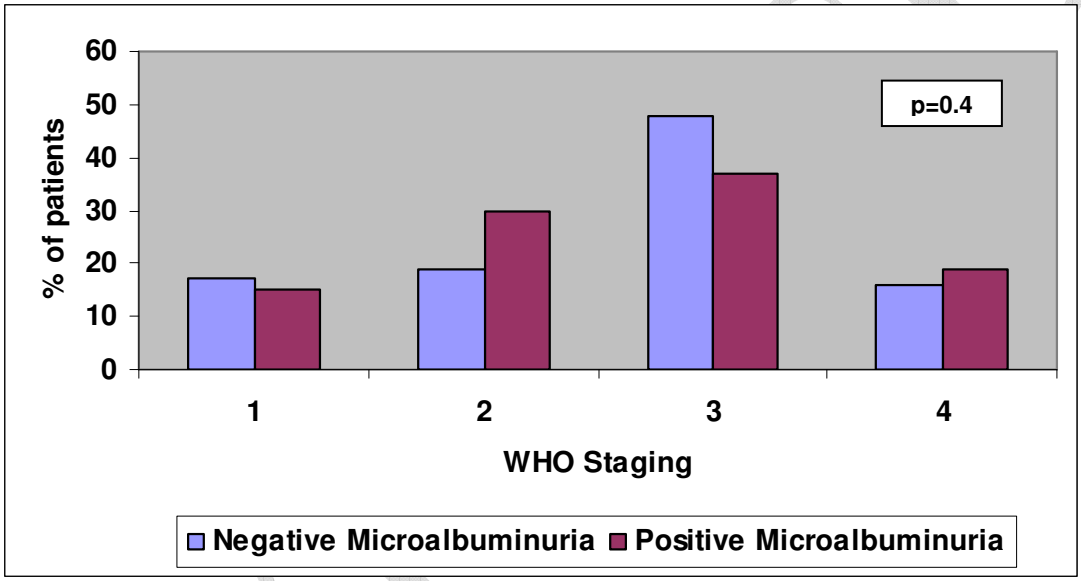

Figure 4 WHO staging characteristics of HIV positive patients with negative and positive microalbuminuria

3.4.1.4 Laboratory parameters

The mean CD4 percentage of HIV seropositive patients with microalbuminuria was 16.8 ± 8 % (1.4-29%) compared to those without microalbuminuria was 20 ± 8 % (1.4-41%), p=0.4. This correlated to a CDC immunological category 2 (moderate immunosuppression).[34] However, statistical significance was only noted in patients with no evidence of immunosuppression (category 1), see figure 5.

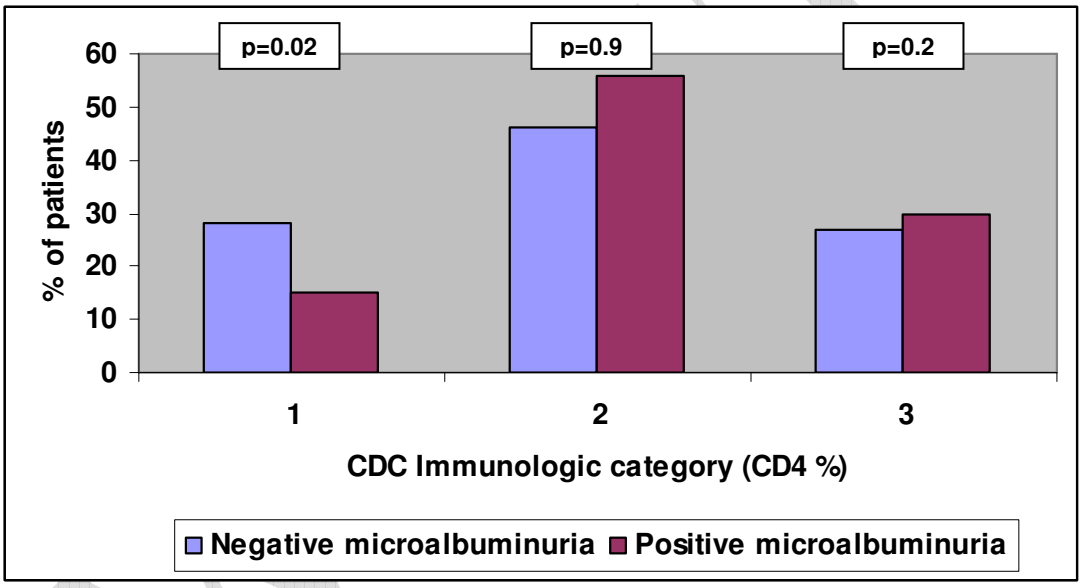

Figure 5 CDC Immunological categories (based on CD4 %) of HIV positive patients with negative and positive microalbuminuria

(CDC immunologic category 1= no, 2= moderate and 3= severe immunosuppression) [34]

The mean CD4 absolute count in HIV positive patients with microalbuminuria (493 ± 330 x 10^6/l) was significantly lower than those without microalbuminuria (780 ± 702 x 10^6/l), p=0.03; especially in immunologic categories 1 and 2, see figure 6. Thus the absolute count maybe a more important factor than percentage CD4 count as in adult HIV disease.[9,25,26]

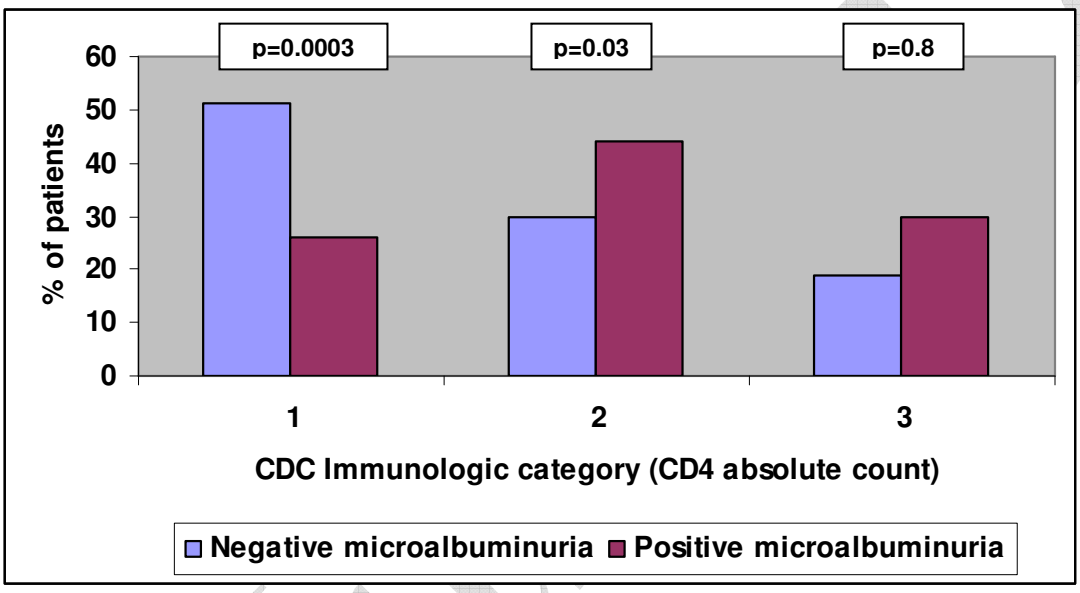

Figure 6 CDC Immunological categories (based on CD4 absolute count) of HIV positive patients with negative and positive microalbuminuria

Therefore, the majority of microalbuminuric patients have moderate to no immunosuppression (CDC immunologic categories 1 and 2) on the basis of CD4 absolute counts and percentages indicating some degrees of preservation of immune function, see figure 7.

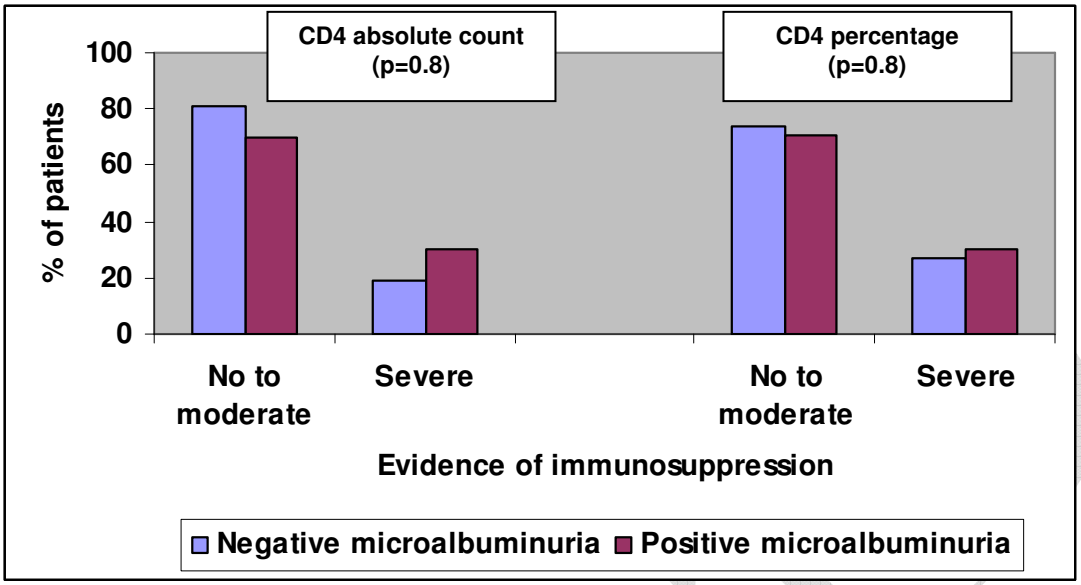

Figure 7 Comparison of CD4 absolute count and percentage (CDC Immunological categories) of HIV positive patients with negative and positive microalbuminuria

The mean viral load for HIV seropositive patients with and without microalbuminuria was 8 ± 18 x 10^5RNA copies/ml (0.006 to 91 x 10^5RNA copies/ml) and 3 ± 7 x 10^5RNA copies/ml (0.003 to 30 x 10^5RNA copies/ml) respectively (p=0.1). The majority of patients with and without microalbuminuria have viral loads <100 000 RNA copies/ml, see table 4. The mean globulin concentration in patients with and without microalbuminuria was 68g/l ± 16 and 53g/l ± 14 respectively (p=0.1). Thus the viral load and high globulins are probably factors not associated with microalbuminuria.

Table 4 Viral load in patients with negative and positive microalbuminuria [35]

Viral load	Microalbuminuria	
	Negative No. (%)	Positive No. (%)
<100 000	53 (50)	16 (15)
≥100 000	25 (24)	11 (10)
P value	0.5	

In patients with HIV, proteinuria was present in 8 (7%) patients and none in 100 (93%) patients. In the absence of the proteinuria, microalbuminuria was present in 21 (19%) patients. Of the remaining 8 patients with proteinuria, 6 (6%) had microalbuminuria and no haematuria, see table 5. Thus microalbuminuria is more sensitive than standard urine dipstick for proteinuria in screening for renal disease in the presence of HIV, p=0.03.

Table 5 Urine dipstick of patients with negative and positive microalbuminuria

		Microalbuminuria	
		Negative n=81 No. (%)	Positive n=27 No. (%)
Proteinuria (p=0.03)	**≤ 20mg/dl**	79 (73)	21 (19)
	> 20mg/dl	2 (2)	6 (6)

In assessing the renal function of patients with microalbuminuria, the mean creatinine was 37 ± 7 (11-70), p=0.08 and the estimated GFR 155 ± 43 ml/min/1.73m^2 ranging from normal to hyperfiltration (80-241 ml/min/1.73m^2), p=0.7. This was similar to patients without microalbuminuria (mean creatinine 35 ± 11 [21-48µmol/l] and estimated GFR 154 ± 62 ml/min/1.73m^2 [71-440 ml/min/1.73m^2]). Hyperfiltration was only noted in malnourished patients, see table 6.

Table 6 Calculated glomerular filtration rate (Schwartz GJ et al[31]) in relation to nutritional status

Nutritional status	**GFR (ml/min/1.73m^2)**				**P value**
	Mean ± SD		**Range**		
	neg MA	**pos MA**	**neg MA**	**pos MA**	
Normal	128*	102*	117-216	84-154	0.3
Moderate malnutrition	0	0	0	0	0
Severe malnutrition	141 ± 36	142 ± 40	71-216	80-223	0

*Median GFR, SD=standard deviation

Classification of malnutrition based on the WHO Technical Report Series[36]

CHAPTER 4

DISCUSSION

Proteinuria is a common feature of HIV infection in children. In our paediatric outpatient HIV unit, all HIV positive patients are screened for renal disease at every follow-up visit by standard urine dipsticks. In the absence of underlying urinary tract infections, patients with persistent proteinuria or hematuria, have further evaluation of renal function by measuring the urea, creatinine and electrolytes. This is followed by measuring urine protein to creatinine ratio and by ultrasound imaging to assess kidney size, as large echogenic kidneys are a feature of HIVAN.

The most salient observation in our study was the association between microalbuminuria and the absence of proteinuria. When screening for renal disease in HIV using standard urine dipsticks, patients with subclinical renal disease will not be detected. Urine dipsticks are poor diagnostic tools for assessing proteinuria as they lack sensitivity and have a low negative predictive value.[37] Although HIVAN can present in asymptomatic HIV infected patients, massive proteinuria and renal insufficiency are late manifestations as evidenced by low number of CD4 cells.[38] With normal renal function and no proteinuria, microalbuminuria was present in our patients with moderate to no immunosuppression.

Thus suggesting that microalbumin assessment of urine is a sensitive screening test compared to standard urine dipsticks however, the specificity of microalbumin testing for HIVAN needs to be evaluated in further paediatric studies in patients with renal biopsy proven HIVAN and serial measurements of microalbuminuria.

HIVAN is possible in patients without overt nephrotic syndrome and even in patients who only have microalbuminuria. Han TM et al measured persistent microalbuminuria in 7(8%) of 90 patients tested. [9] Interestingly of the 7 patients, 6 patients were biopsy proven HIVAN with normal renal function and the mean urine protein excretion was 0.14g/24hr, thus indicating that microalbuminuria is an early indicator of subclinical renal disease in HIV. Similarly, microalbuminuria has been evaluated by many investigators to diagnose subclinical renal involvement in other systemic diseases, eg. systemic lupus erythematosus and diabetes mellitus with renal involvement.

Valente de AR et al performed renal biopsies in 30 patients with systemic lupus erythematosus with microalbuminuria but no clinical signs of renal involvement. Fifteen (50%) of cases had mesangial glomerulonephritis (MGN) type 2b, 12 (40%) had MGN type 2a and 3 (10%) showed no changes on light microscopy or immunofluorescence.[39] Similarly, a significant number of microalbuminuric diabetic patients had diabetic nephropathy and no overt proteinuria.[40]

The presence of microalbuminuria in the face of proteinuria suggests mainly the excretion of albumin. This may be due to the pathophysiological mechanism of increased glomerular capillary wall permeability from charge selectivity [41] in HIVAN.

When profiling for proteinuria in paediatric patients with any chronic disease, the percentage of albuminuria is greatest in those with glomerular disease as compared to those with tubular dysfunction.[37] Over 80% HIV positive patients with subclinical renal disease have glomerular permeability defects or tubular dysfunction.[42]

The prevalence of microalbuminuria; based on a single morning urine sample in our HIV infected children prior to commencing antiretroviral therapy, was 25%. This association between HIV and microalbuminuria has been observed in adults, with prevalence rates varying between 8.7% to 30%.[9,25,26,43] However, to date no paediatric studies have been undertaken to assess this association.

In our study, HIV infected children with microalbuminuria were older than 5 years of age with clinical stage 2 and 3 disease. Kimmel PL et al have showed that microalbuminuria is not related to the staging of HIV infection.[43]

In 2 published adult studies, CD4 count >200 cells/mm^3 have been associated with microalbuminuria.[9,25] Similarly in our study majority of patients had evidence of moderate immunosuppression with some preservation of immune function. This is in contrast to the findings by Szczech L et al, where microalbuminuric patients had mean CD4 counts <200cells/ml.[26]

The CD4 cell counts in children are much higher than adults especially in early childhood.[44] In the first few months after birth the CD4 counts may be above 200 cells/mm^3 (compared to 500-1200 cells/mm^3 in uninfected adults) and it declines as the child gets older; reaching adult levels in late childhood (by age 6 years). In contrast, the CD4 count and percentage are stable throughout adulthood. But absolute counts correlate better with microalbuminuria as shown in our study. However, the use of CD4 percentage over absolute counts in children is recommended to guide and monitor HIV treatment as it is relatively stable in young children.

When comparing the clinical characteristics of patients with microalbuminuria, there was no significant difference in age, gender, nutritional status, clinical presentation, CD4 percentage, viral load or renal function, this is similar to findings by Baekken M et al.[45] Although LIP and tuberculosis are commonly associated with HIV, according to Kala U et al,[17] there is a good correlation between LIP and immune complex mediated HIVAN.

In other adult studies risk factors for microalbuminuria varied according to the study design. Urinary microalbumin levels correlated with CD4 cell counts, white blood cell counts and clinical AIDS in a study by Luke DR et al.[25] Szeczech LA et al demonstrated that CD4 lymphocyte counts <200 cells/mm^3, viral load together with hypertension and insulin resistance predicted the severity of microalbuminuria in HIV.[26] Larger studies with serial microalbumin measurements and correlation with renal histology are required to differentiate better the combination of the various factors causing microalbuminuria.

Limitations

It is important to note the limitations of this study.

- The true prevalence of microalbuminuria in our study may be an overestimation. Single urine samples were taken, thus the change in microalbuminuria could not be assessed. Although albumin excretion is variable on a daily basis there is increasing evidence that persistent proteinuria is a marker of both renal disease and progressive renal injury.[19] In diabetes, 40-50% of initial microalbuminuria progress to persistent, 30-60% show intermittent microalbuminuria, while 20% never develop microalbuminuria. The prognostic value of a single sample with microalbuminuria in diabetes is of limited value. The positive predictive and negative predictive values are 76% and 99.5% respectively.[46] Abitbol CL et al also noted that the degree of proteinuria correlated with loss of renal function and progression to ESRD, in chronic kidney disease of any cause in children.[37] The above may apply to HIV, however further surveillance and studies are required.

- Although the relationship between HIVAN and microalbuminuria could not be evaluated by renal biopsy in this population, adult studies have shown that HIVAN is associated with microalbuminuria. In a study by Han TM et al, in 7 patients with persistent microalbuminuria, 6 had HIVAN and 1 interstitial nephritis.[9]

  In another study, 107 HIV patients that underwent renal biopsy, 52 (49%) presented with non nephrotic proteinuria and 55 (51%) nephrotic range proteinuria. Of the patients with non nephrotic proteinuria, HIVAN was confirmed in 11 (21%) patients while 41 (79%) showed non HIV associated nephropathy.[47]

- The evaluation of renal function by calculated GFR may not reflect true renal function; especially in children with low muscle mass because the constant k tends to be lower in this subset of patients. In malnourished children methods for measuring GFR are required as there are no studies that have validated approaches using plasma creatinine and anthropometric measurement of muscle mass.[31]

- There were more HIV positive patients than negative, thus selection bias cannot be completely excluded.

Strengths

This is the first prospective study to assess microalbuminuria in black South African HIV positive children as a screening test for early renal involvement.

Clinical relevance and recommendations

With the increasing prevalence of HIV and HIV related complications, the HIV Medicine Association of America have recommended screening for kidney disease in HIV infected individuals.[48] Just as the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDQI) guidelines for diabetes,[22] uses microalbuminuria to screen for early renal disease, so to should this screening test be implemented in HIV. Also, spot albuminuria should be used in screening for renal disease in paediatrics as significant proteinuria can be missed in older children.[37]

CHAPTER 5

CONCLUSION

Microalbuminuria is prevalent in HIV seropositive children especially those over 5 years of age without clinical evidence of any AIDS defining illnesses. Majority of microalbuminuric patients have moderate to no evidence of immunosuppression. Absolute CD4 counts appear to better correlate with microalbuminuria than CD4 percentage. In the absence of proteinuria; microalbuminuria does indicate subclinical renal disease; however, it is a not valid diagnostic test for HIVAN as yet. Renal biopsy still remains the gold standard for diagnosing HIVAN. A renal biopsy may be indicated in those patients presenting with persistent microalbuminuria with normal renal function. Early screening for microalbuminuria in HIV positive patients when immune function is still preserved may possibly prevent both progression of renal disease and underlying HIV with early initiation of antiretroviral therapy.

Future studies that can arise from this study:

- To assess the association between microalbuminuria and biopsy proven nephropathy in HIV positive children.
- Repeat sampling of microalbuminuria in HIV infected children to assess the true prevalence and/ or persistence of microalbuminuria.
- To assess calculated GFR in malnourished HIV positive and negative children by radioisotopes and to determine a possible correction factor in these patients that can be used in the calculation of GFR utilizing the Schwartz formula.

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APPENDIX A

Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mistry

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M050808**
PROJECT	Relevance of Microalbumuria in Screening for HIV-Associated Nephropathy
INVESTIGATORS	Dr BJ Mistry
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	05.08.26
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 05.09.27 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof U Kala

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES