THE PREVALENCE OF MICROALBUMINURIA IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS AT THE OUTPATIENT CLINIC IN KENYATTA NATIONAL HOSPITAL

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE DEGREE OF MASTER OF MEDICINE IN PAEDIATRICS AND CHILD HEALTH AT THE UNIVERSITY OF NAIROBI

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DECLARATION

This dissertation is my original work and has not been submitted for a degree in any other university

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DEDICATION

I dedicate this work to my parents Dr. Michael O. Amolo and Mrs. Nereah A. Amolo, and to my brother Steve for their unwavering support, understanding and encouragement during all these years of study.

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ABBREVIATIONS

AGEs	advanced glycosylation end-products
ATII	angiotensin II
BM	basement membrane
BMI	body mass index
CDC	Centre for Disease Control and Prevention
CI	confidence interval
DCCT	Diabetes Control and Complications Trial
DKA	diabetic ketoacidosis
EDTA	ethylenediaminetetraacetic acid
ESRD	end stage renal disease
GFR	glomerular filtration rate
HbA_{1c}	glycosylated hemoglogin type A_{1c}
IQR	interquartile range
KES	Kenya Shillings
LDL	low density lipoprotein
SD	standard deviation
PDGF	platelet-derived growth factor
TGF-beta	transforming growth factor-beta
VEGF	vascular endothelial growth factor

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ABSTRACT

OBJECTIVE: The purpose of this study was to determine the prevalence of microalbuminuria, and to relate it to sociodemographic features and glycemic control, among children and adolescents with type 1 diabetes mellitus.

RESEARCH DESIGN: This was a hospital- based prospective cross-sectional study.

SUBJECTS: The study included 65 children (31 male) with a mean age of 10.9 ± 4.5 years attending the Kenyatta National Hospital diabetic outpatient clinic in Nairobi between June 2009 and January 2010. The median duration of diabetes (IQR) among the subjects was 2 (1-4) years.

METHODS: A structured questionnaire was used for evaluation of sociodemographic data. Urine and blood samples were collected and analysed for urine albumin-to-creatinine ratio using the CLINITEK Urine Chemistry Analyser, and Hemoglobin A_{1c} using the Hemoglobin A_{1c} Immunoturbidimetric test, respectively. Persistent microalbuminuria was defined as a urine albumin to creatinine ratio of 30-299μg/mg on at least two occasions within a 3 to 6 month period. Subjects with a urine albumin to creatinine ratio of 20-29μg/mg on at least two occasions within a 3 to 6 month period were categorized as being at risk of developing microalbuminuria. Other information obtained included age, gender, duration of diabetes, body mass index, blood pressure, economic status, level of education and knowledge of the patient and caregiver.

RESULTS: The prevalence of persistent microalbuminuria was 6.2%, while another 6.2% of the subjects had overt proteinuria. Three (4.6%) subjects were at risk of developing microalbuminuria. No significant differences in sociodemographic features and glycemic control were found among patients with persistent microalbuminuria compared with those without persistent microalbuminuria.

CONCLUSION: A prevalence of persistent microalbuminuria of 6.2% was found in this population of children and adolescents with type 1 diabetes mellitus. These children and adolescents may thus be at particular risk of cardiovascular morbidity and mortality, as well as later end-stage renal disease. This study detects, however, a much lower prevalence than reported in previous studies from sub-Saharan Africa.

1. LITERATURE REVIEW

Introduction

Diabetes mellitus is a common, chronic, metabolic syndrome characterized by hyperglycemia as a cardinal biochemical feature. There are two major forms of diabetes mellitus. Type 1 diabetes results from deficiency of insulin secretion due to destruction of the pancreatic beta cells whereas type 2 diabetes is a consequence of reduced beta cell function with insulin resistance occurring at the level of skeletal muscle, liver and adipose tissue.

Type 1 diabetes is the most common endocrine-metabolic disorder of childhood and adolescence, with important consequences for physical and emotional development. Patients with type 1 diabetes have little or no pancreatic function, a tendency to develop ketoacidosis and a dependency on exogenous insulin to sustain life.

Epidemiology

In the second edition of the International Diabetes Federation's Diabetes Atlas, it is estimated that 194 million people had diabetes mellitus in the year 2003, and about two-third of these people lived in developing countries (IDF 2003). It was also estimated that 430,000 children around the world have diabetes, with 65,000 new cases diagnosed each year.[1] Type 1 diabetes accounts for more than 90% of childhood and adolescent diabetes in most western countries.

There are few published studies describing the incidence and prevalence of type 1 diabetes in Sub-Saharan Africa. An annual incidence ranging from 1.5 per 100,000 (Tanzania)[2] to 10.3 per 100,000 (Sudan)[3] and a prevalence ranging from 0.3 per 1,000 (Nigeria)[4] to 0.95 per 1,000 (Sudan)[3] has been reported. A rise in incidence has been noted in many countries, with reports of a disproportionately greater increase in those younger than 5 years.[5] Gender differences in incidence have also been noted in some populations.[5]

Microvascular complications

The long-term microvascular complications include retinopathy, nephropathy and neuropathy. Persistent hyperglycemia leads to irreversible covalent bonding between glucose and sialic acid molecules within the vascular walls. The negative charge which results from acidification enhances atherosclerosis with consequent narrowing of low calibre vessels and compromised blood supply to the affected structures.

Although diabetes is associated with derangement in protein and lipid metabolism, the control of blood glucose is the most important intervention in the prevention of long term complications. A landmark study, the 1993 Diabetes Control and Complications Trial (DCCT) established conclusively the association between higher glucose levels and long-term microvascular complications. Intensive glycemic control produced dramatic reductions of retinopathy, nephropathy and neuropathy by 47-76%.[6]

A declining incidence of complications has been reported in many areas with specialized clinics[7] where there have been improvements in the management of diabetes, identification of risk factors and regular screening for complications. However, in areas where health care is not optimal, as is the case in sub-Saharan Africa, a greater risk of complications still remains.

Diabetic nephropathy is the major cause of morbidity and mortality among young patients with type 1 diabetes mellitus. In a study done in Soweto, South Africa on mortality and outcome of type 1 diabetes between 1982 and 1992, 50% of all causes of mortality were due to due to renal failure.[8]

Diabetic nephropathy

Diabetic nephropathy occurs in children who have poorly controlled diabetes. It is a clinical condition characterized by persistent proteinuria, decline in glomerular function, hypertension and progression to end stage renal disease.

The following risk factors have been found to be associated with diabetic nephropathy: poor glycemic control,[9] high blood pressure,[10,11] duration of disease,[12–14] smoking,[7] dyslipoproteinemia,[7] higher body mass index,[7] age at diagnosis (later onset with younger age at diagnosis),[15] female gender[15] and family history of renal disease or hypertension.

The exact cause of diabetic nephropathy is unknown, but there are various postulated mechanisms. Hyperglycemia may directly result in mesangial expansion and injury by an increase in the mesangial cell glucose concentration. Glucose can also bind irreversibly to proteins in the kidneys and circulation to form advanced glycosylation end-products (AGEs), which form complex cross-links over years of hyperglycemia and cause stimulation of growth and fibrotic factors via receptors for AGEs.

Mediators of proliferation and expansion, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta) and vascular endothelial growth factor (VEGF) that are elevated in diabetic nephropathy can contribute to further renal and microvascular complications.

Angiotensin II (ATII) contributes to the progression of diabetic nephropathy. It is stimulated in diabetic nephropathy despite the high volume state typically seen with the disease. It preferentially constricts the efferent arteriole in the glomerulus, leading to higher glomerular capillary pressures. It also stimulates renal growth through ATII type 1 receptors, which secondarily upregulate TGF-beta and other growth factors.

Clinical presentation and progression of diabetic nephropathy

Diabetic nephropathy in patients with type 1 diabetes progresses through five predictable stages (see Table 1). Stage 1 is usually present at the time of diagnosis and is characterised by glomerular hyperfiltration due to renal hyperfunction and hypertrophy. The albumin excretion rate may be normal or increased.

Stage 2 (silent stage) is characterised by progression of glomerular damage resulting in a thickened basement membrane and an expanded mesangium. The glomerular filtration rate at this stage has returned to normal and albumin excretion is normal.

Stage 3 (incipient nephropathy) is characterized by a falling glomerular filtration rate and microalbuminuria (albumin excretion 30-300mg/day). Stage 4 (overt or dipstick positive nephropathy) is characterised by macroalbuminuria (albumin excretion > 300mg/day or > 200μg/ min) and a glomerular filtration rate that is below normal. Hypertension is invariably present at this stage. By the final stage (end stage renal disease), the glomerular filtration rate has fallen to below 10ml/min and renal replacement therapy is required.

Table 1.

Natural History of Diabetic Nephropathy

	Designation	Characteristics	GFR (minimum)	Albumin Excretion	Blood Pressure	Chronology
Stage 1	Hyperfunction and hypertrophy	Glomerular hyperfiltration	Increased in type 1 and type 2	May Be Increased	Type 1 normal Type 2 normal hypertension	Present at time of diagnosis
Stage 2	Silent stage	Thickend BM Expanded mesangium	Normal	Type 1 normal Type 2 may be <30-300 mg/d	Type 1 normal Type 2 normal hypertension	First 5 years
Stage 3	Incipient stage	Microalbuminuria	GFR begins to fall	30-300 mg/d	Type 1 increased Type 2 normal hypertension	6-15 years
Stage 4	Overt diabetic nephropathy	Macroalbuminuria	GFR below N	>300 mg/d	Hypertension	15-25 years
Stage 5	Uremic	ESRD	0-10	Decreasing	Hypertension	25-30 years

Microalbuminuria

The clinical evidence of nephropathy is the appearance of low but abnormal levels of albumin in urine (microalbuminuria). The term microalbuminuria denotes increases in albumin excretion rates outside of the normal range, but too low to register on the normal clinic albusticks (see Table 3). Microalbuminuria is confirmed by at least two positive results over a period of 3 to 6 months. Patients with microalbuminuria are referred to as having incipient nephropathy. Without specific interventions, about 80% of patients with type 1 diabetes who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of approximately 10-20% per year to the stage of overt nephropathy or macroalbuminuria over a period of 10-15 years, with hypertension also developing along the way. The rate increases quite dramatically during puberty.[16] However, there are reports of diabetic children who develop diabetic nephropathy in prepubertal period.[9,12,15,17_19] These suggest that children with diabetes are at risk of developing increased albumin excretion before puberty and this would warrant regular screening.

Once overt nephropathy occurs, without specific interventions, the glomerular filtration rate gradually falls over a period of several years at a rate that is highly variable from individual to individual (2-20ml/min/yr). End stage renal disease develops in 50% of type 1 diabetic individuals with overt nephropathy within 10 years and in 75% by 20 years.

In addition to being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with either type 1 or type 2 diabetes. Thus the finding of microalbuminuria is an indication for screening for possible vascular disease and aggressive intervention to reduce all cardiovascular risk factors (for example, lowering LDL cholesterol, cessation of smoking, anti-hypertensive therapy).

Prevalence studies on microalbuminuria report figures ranging from 4.8 to 29.3%, and are summarized in Table 2.

Table 2. Prevalences of microalbuminuria in children and adolescents with type 1 diabetes mellitus

Study	Number of patients	Age (years)	Urine sample	Type of study	Prevalences (%)
Mathiesen et al,[20] 1986, Denmark	97	7-18	2 timed overnight	Cross-sectional	20
Schultz et al,[15] 1985-96, UK	514	<16	3 early morning or timed overnight	Longitudinal (cohort)	4.8
MIDAC,[9] 1997-98, UK/ Ireland	1007	10-20	3 early morning	Cross-sectional	9.7
GECER group,[21] 2000, France	702	14.3 ± 2.9	2-3 timed	Cross-sectional	5.1 ± 1.6
Donaghue et al,[22] 1989-04, Australia	972	Median (IQR) 12 (11.5-14.4)	3 timed overnight	Longitudinal (cohort)	4.6
Moayeri et al,[23] 1997-04, Iran (Tehran)	118	5-20	3 timed	Longitudinal	19.5
Lutale et al,[24] 2003-04, Tanzania (Muhimbili)	91	4-44.8	2 timed overnight	Cross-sectional	12
Gallego et al,[25] 2006, Australia	955	<16	3 timed overnight	Prospective	13.4
Abdeyazdan et al,[26] 2006, Iran (Isfahan)	39	<21	One 24-hour	Cross-sectional	23.1
Majaliwa et al,[27] 2005-06, Tanzania (Muhimbili)	99	5-18	One MICROTEX	Cross-sectional	29.3
Zahra et al,[28] 2005-07, Iran (Hamedan)	105	13.3 ± 5.5	One 24-hour	Prospective	14.3

Screening for albuminuria

Screening for microalbuminuria can be performed by three methods:
1) measurement of the albumin-to-creatinine ratio in a random spot collection,
2) 24-hour collection with creatinine, allowing the simultaneous measurement of creatinine clearance, and
3) timed (for example, 4-hour or overnight) collection.

The first method is often found to be the easiest to carry out in an office setting, generally provides accurate information, and is therefore preferred; first-void or other morning collections are best because of the known diurnal variation in albumin excretion, but if this timing cannot be used, uniformity of timing for different collections in the same individual should be employed. This method, however, does not account for patient weight or muscle mass. The timed urine collections needed to estimate albumin excretion rates are often impractical, particularly during childhood.

Microalbuminuria is said to be present if urinary albumin excretion is 30-300 mg/24 hours (equivalent to 20-200 μg/min on a timed specimen or 30-300 mg/g creatinine on a random sample) (see Table 3). Short-term hyperglycemia, exercise within 24 hours, urinary tract infections, hematuria, marked hypertension, heart failure, and acute febrile illness can cause transient elevations in urinary albumin excretion.

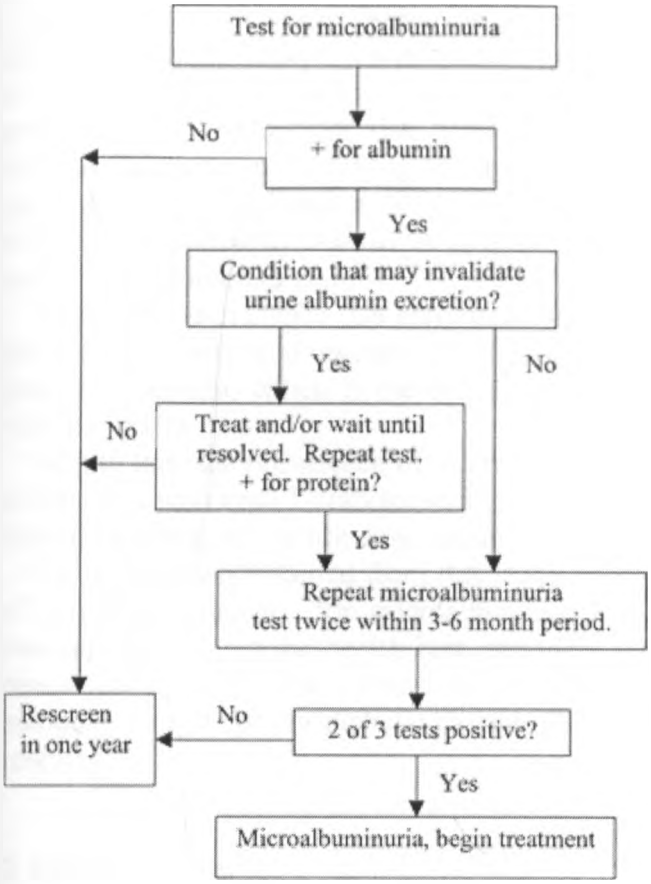
Table 3. Definitions of abnormalities in albumin excretion

Category	Spot collection/ albumin creatinine ratio (μg/mg creatinine)	24-h collection (mg/24 h)	Timed collection (μg/min)
Normal	<30	<30	<20
Microalbuminuria	30–300	30–300	20–200
Macroalbuminuria	>300	>300	>200

If assays for microalbuminuria are not readily available, screening with reagent tablets or dipsticks for microalbuminuria may be carried out, since they show acceptable sensitivity (95%) and specificity (93%) when carried out by trained personnel. Because reagent strips only indicate concentration and do not correct for creatinine as the spot urine albumin-to-creatinine ratio does, they are subject to possible errors from alterations in urine concentration. All positive tests by reagent strips or tablets should be confirmed by more specific methods.

There is also marked day-to-day variability in albumin excretion, so at least two of three collections done within a 3 to 6 month period should show elevated levels before designating a patient as having microalbuminuria. An algorithm for microalbuminuria screening is given in Figure 1.

Figure 1. Screening for microalbuminuria



Treatment of diabetic nephropathy

Diabetic nephropathy is the leading cause of chronic renal disease in the western world. It is also one of the most significant long-term complications in terms of morbidity and mortality for individual patients with diabetes.[29_31]

Recent studies have demonstrated that the onset and course of diabetic nephropathy can be ameliorated to a very significant degree by several interventions, but these interventions have their greatest impact if instituted at a point very early in the course of the development of this complication. [29_31]

Once albuminuria is diagnosed, a number of factors attenuate the effect of hyperfiltration on the kidneys:

- Meticulous control of hyperglycemia
- Aggressive control of systemic blood pressure
- Selective control of arteriolar dilation by use of angiotensin-converting enzyme inhibitors (thus decreasing transglomerular capillary pressure)
- Dietary protein restriction (because high protein intake increases renal perfusion rate).

2. STUDY JUSTIFICATION AND UTILITY

In a study conducted by Ngwiri et al between May and October 2003 in three outpatient clinics in Nairobi, Kenya, it was found that the prevalence of poor glycemic control was high at 72% and majority of patients were at high risk for developing complications. Adolescents were found to be a high risk group for poor control of type 1 diabetes. One of the recommendations was that a study be carried out to determine the prevalence of microvascular complications in our pediatric diabetic patients. With such poor glycemic control as reported by Ngwiri et al in our set up, we expect diabetic nephropathy to occur earlier than in in other centres.

There is good evidence that early treatment halts the progression from microalbuminuria to macroalbuminuria and eventually end stage renal disease.[29–31] Therefore, detection of microalbuminuria, which is the earliest manifestation and treatable form of nephropathy, is very important.

Despite this, the prevalence of microalbuminuria and its correlates in Kenyan children and adolescents with type 1 diabetes mellitus has not been evaluated. Furthermore, evaluation for diabetic nephropathy is not done routinely.

The information obtained from this study will guide recommendations for the development of screening practices for microalbuminuria. It will also be used to make appropriate recommendations to the health care providers and primary care givers to enhance quality of care to the study subjects. The results will be useful, not only in improving the management and quality of life of our diabetic patients, but also in serving as a basis for appropriate further research.

3. OBJECTIVES

Primary Objective

- To determine the prevalence of microalbuminuria in children and adolescents with type 1 diabetes mellitus at the Kenyatta National Hospital outpatient clinic.

Secondary Objective

- To determine the factors associated with microalbuminuria in children and adolescents with type 1 diabetes mellitus (including sociodemographic characteristics, age of the patient, gender, duration of disease, knowledge of the disease and glycemic control).

Although this study does not have the power to subanalyse, the findings will be used to make recommendations for further research to analyse these factors.

4. STUDY METHODOLOGY

Study Design

This was a hospital-based prospective cross-sectional study.

Study Site

The study was carried out at the Kenyatta National Hospital diabetic clinic, which has about 70 children attending the clinic. This is a government and national referral hospital which also serves the population within Nairobi Province. It is also a teaching hospital for the University of Nairobi.

Study Period

The study was conducted between June 2009 and January 2010.

Study Population

This was children aged 1-19 years presenting at the Kenyatta National Hospital diabetic clinic, diagnosed as having type 1 diabetes mellitus (see study definitions) on the basis of the World Health Organization and American Diabetes Association criteria. These are:

- Fasting plasma glucose >7.0 mmol/L or
- Fasting whole blood glucose >6.1 mmol/L or
- 2hr post-glucose plasma glucose >11.1mmol/L or
- 2hr post-glucose whole blood glucose >10.0 mmol/L or
- Random plasma glucose >11.1 mmol/L

On more than one occasion.

Patient Selection

(i) Inclusion Criteria:

- Male and female children aged 1-19 years with a diagnosis of type 1 diabetes mellitus.
- Signed informed consent to participate in the study (see Appendix B) from the study subject if 18 years and above. For children below 18 years consent was obtained from the parent/guardian together with assent from the child if above 7 years.

(ii) Exclusion Criteria:

- Patients on nephrotoxic chemotherapy.
- Patients with known renal disease other than that caused by diabetes mellitus.
- Patients with end stage renal disease.

Sampling Technique

Consecutive patients who satisfied the inclusion criteria during the study period were recruited into the study.

Sample Size Calculation

Sample size was calculated using Fischer's formula

$$\mathbf{n = \frac{Z^2_{\alpha/2} \times P(1-P)}{d^2}}$$

where

n = sample size

$Z^2_{\alpha/2}$ = the corresponding value to the 95% confidence level (1.96)

d = absolute precision (5%)

p = known prevalence from other studies

The prevalences of microalbuminuria range from 5.1% (GECER study group, France) to 29.3% (Majaliwa, Tanzania).

Therefore $n = \frac{1.96^2 (0.051) (0.949)}{0.0025}$

n = 74 if the prevalence in France is used for estimation

or $n = \frac{1.96^2 (0.293) (0.707)}{0.0025}$

n = 318 if the prevalence in the Tanzania study is used for estimation

Since the target population is small (< 5,000), the sample size can be reduced because a given sample size provides proportionately more information for a small population than for a large population. The sample size is adjusted to get the new sample size (n') using the **Finite Population Correction (FPC)**

$$\mathbf{n' = \frac{n}{1 + \frac{n-1}{N}}}$$

where

n' = the sample size when the population is less than 5,000

n = the sample size when the population is greater than 5,000

N = the total size of the population from which a sample will be drawn for the survey.

The target population for this study was 70 children (Kenyatta National Hospital Medical Records, 2009).

Therefore $$n' = \frac{74}{1 + \frac{74-1}{70}}$$

n' = 37 if the prevalence in France is used for estimation

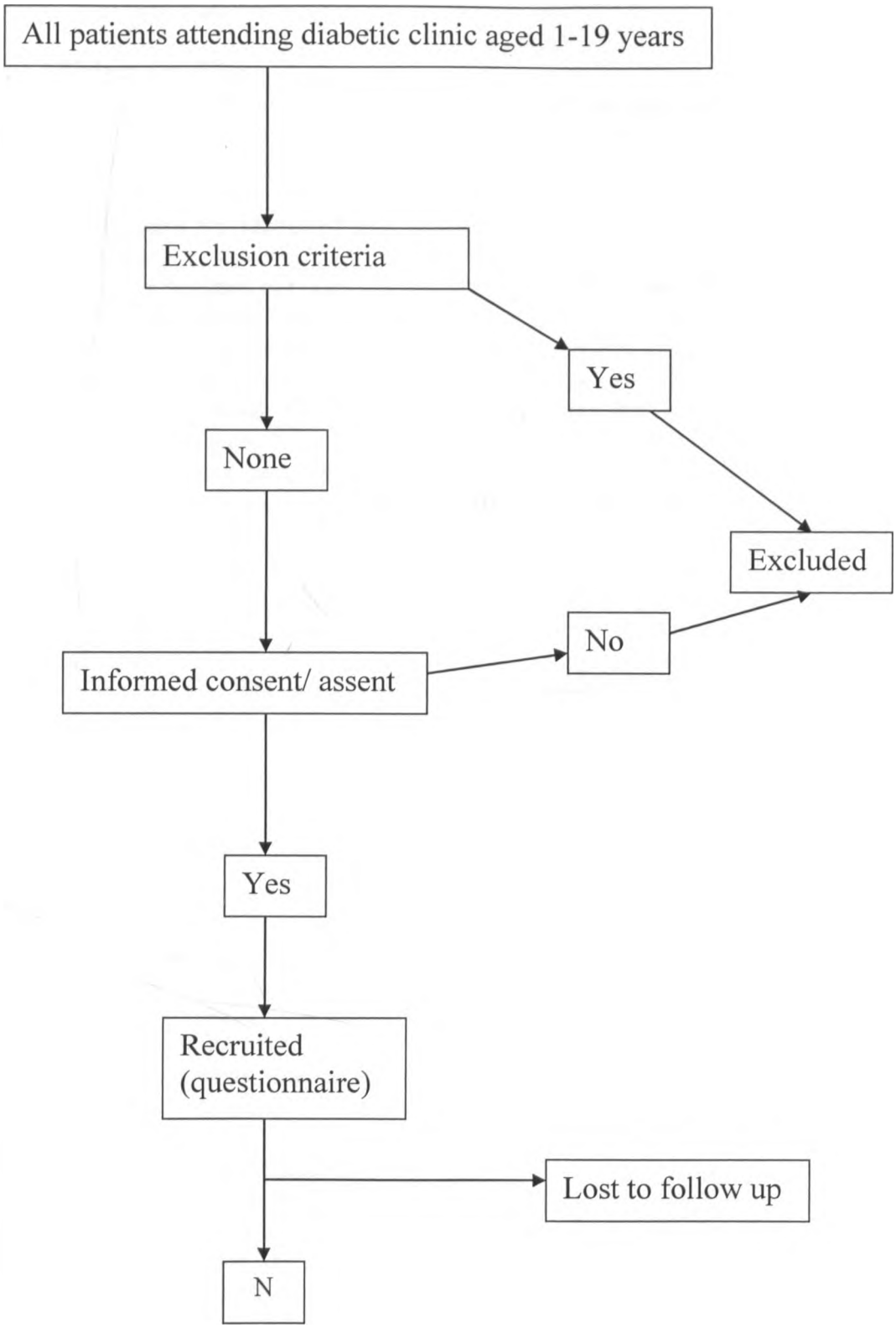
or $$n' = \frac{318}{1 + \frac{318-1}{70}}$$

n' = 58 if the prevalence in the Tanzania study by Majaliwa et al is used for estimation

A minimum sample size of 58 was targeted for this study.

Screening and recruitment

Figure 2. Flow chart of screening and recruitment



5. STUDY DEFINITIONS

(i) Adolescence

This is the time between the beginning of sexual maturation (puberty) and adulthood. Adolescence is roughly considered to be the period between 10 and 19 years of age. This age range was used to define adolescence in this study.

(ii) Type 1 diabetes mellitus

Onset of diabetes in childhood with ketoacidosis and insulin dependency has traditionally been sufficient to diagnose type 1 diabetes, while onset in older, obese patients with primary insulin resistance suggested type 2 diabetes. Unfortunately, features of type 1 and type 2 diabetes may be present in the same patient, making differentiation difficult. No diagnostic studies in the literature are identified that definitively demonstrate how to separate type 1 from type 2 diabetes.

A patient's age may suggest, but does not reliably distinguish, diabetes types. In fact, newly diagnosed 12-year-old children have an equal incidence of type 1 as type 2 diabetes.

A history of diabetic ketoacidosis (DKA) also does not reliably distinguish between types 1 and 2. A retrospective chart review gathered data on adults over 18 years of age who were admitted for DKA in an urban US hospital. Many patients with DKA were subsequently diagnosed with type 2 diabetes.[32]

The need for insulin may be used to determine whether a patient has type 1 or type 2 diabetes (which is needed from the beginning in type 1, and less commonly early in type 2). Studies suggest that a majority of patients with type 2 diabetes would require some form of exogenous insulin therapy after a duration of 8 to 10 years of their disease.

Although C-peptide levels (a measure of endogenous insulin production), autoantibodies, and adiponectin-to-leptin ratios show some utility, they do not yet have a standard diagnostic role. The overlapping presence of autoantibodies in both types of diabetes limits their use.

In this study, the onset of diabetes in childhood and adolescence, insulin dependency from the time of diagnosis and the absence of obesity was used to diagnose type 1 diabetes mellitus.

6. DATA COLLECTION

Clinical methods

Eligible patients were enrolled in the study by entering their names into a register book and were allocated a code number. Sociodemographic data, specifically relating to age and gender of the patient, level of education of both the patient and the parent/guardian and whether in paid employment (parent/guardian) or not was obtained using a structured questionnaire (see Appendix A).

A complete medical history was obtained and a comprehensive medical examination undertaken. Height was determined to the nearest 5mm with a rigid stadiometer against a vertical wall. Weight was measured without shoes or heavy clothing to the nearest 50g using an electronic scale. Body mass index was calculated according to the Quetelet equation (weight in kilograms divided by the square of the height in metres). Body mass index percentiles were determined based on age and sex using the CDC 2000 BMI for age charts (Table 4). Blood pressure was measured by auscultation after 10 minutes of rest using a standard sphygmomanometer with an appropriate paediatric cuff.

Patient clinical records were referred to for information on history of renal disease that is related to diabetes or otherwise. A questionnaire (see Appendix A) was used to obtain information on knowledge of caregivers on management of children and adolescents with type 1 diabetes mellitus.

Table 4. BMI for Age weight status categories based on CDC BMI for Age growth charts

Weight Status Category	Percentile Range
Underweight	< 5th percentile
Healthy weight	5th to < 85th percentile
At risk of being overweight	85th to < 95th percentile
Overweight	≥ 95th percentile

Laboratory methods

Screening for microalbuminuria was performed by measurement of the albumin-to-creatinine ratio in at least 2 spot urine specimens collected over a period of 3 to 6 months. Midstream urine specimens of 10ml each were collected aseptically, between 8 a.m and 12 noon, during consecutive visits, into clean, dry containers. For children who were between 1 and 2 years, or who were not toilet-trained, urine was collected using a plastic urine bag that sticks onto the skin for both males and females. Uniformity of timing for different collections in the same individual was employed due to diurnal variation in albumin excretion.

Testing was done within 2 hours after voiding, otherwise the specimen was stored at 0-8^{0}C for not more than a week. Urine was screened for protein using dipstick analysis. Urines that were positive (macroalbuminuria) or that were visibly bloody were not tested for microalbuminuria.

CLINITEK Microalbumin Reagent Strips are firm plastic strips that contain 2 reagent areas that test for albumin and creatinine in urine. An albumin-to-creatinine ratio is also determined. In this study, CLINITEK Microalbumin Reagent Strips were dipped into the urine and then placed into the CLINITEK 50 Urine Chemistry Analyser test table. The albumin-to-creatinine ratio was then displayed. Microalbuminuria was indicated as a ratio result of 30-300 mg/g. Microalbuminuria was considered present if positive in at least 2 urine specimens. Quality control was ensured by testing with commercially available negative and positive controls that included values for microalbumin and creatinine.

100µl of a venous blood sample was collected from the patient after cleaning the site with alcohol. This sample was collected into a tube containing EDTA. A hemolysate was prepared by dispensing 1ml of Hemolysis Reagent into a tube and mixing this with the 100µl of whole blood. This mixture was allowed to stand for 5 minutes or until complete lysis was evident. The same procedure was done with controls. The HbA_{1c} level was then determined using the Hemoglobin A_{1c} Direct Immunoturbidimetric (latex) test. This method utilizes the interaction of antigen and antibody to directly determine the HbA_{1c} in whole blood. The amount of agglutination is proportional to the amount of HbA_{1c} absorbed onto the surface of latex particles. The amount of agglutination is measured as absorbance.

7. DATA MANAGEMENT AND STATISTICAL ANALYSIS

The data was entered in a statistical program Statistical Product and Service Solutions (SPSS) version 17 and analyzed accordingly. Chi-square tests were used to compare categorical variables and proportions across groups. Continuous variables were compared using student t-test and Mann-Whitney U test. A p value of less than 0.05 was considered statistically significant.

8. ETHICAL CONSIDERATIONS

(i) The study was conducted after written approval by the Department of Paediatrics and Child Health, University of Nairobi, and the Kenyatta National Hospital Scientific and Ethical Review Committee.

(ii) The study was carried out on children whose parents/guardians had given informed consent.

(iii) Information gathered was treated with confidentiality.

(iv) Laboratory results were availed to the clinician taking care of the patient for patient management.

(v) There was no added cost to the patient.

9. RESULTS

A total of 66 participants were enrolled into the study and one was lost to follow-up. Among the remaining 65, 47.7% were male and 52.3% were female (male: female 0.9:1). The age of the participants ranged between 1 and 18 years with a mean ± SD age of 10.9 ± 4.5 years. The median (IQR) duration of diabetes illness among the study participants was 2 (1 to 4) years. Table 4 summarizes the baseline demographic characteristics of all the children. Forty-three percent of the children had a family history of diabetes mellitus and 50.8% had good glycemic control defined by HbA_{1c} levels less than 8%. The median monthly household income was KES 11000.

Table 5. Baseline characteristics of subjects recruited into the study

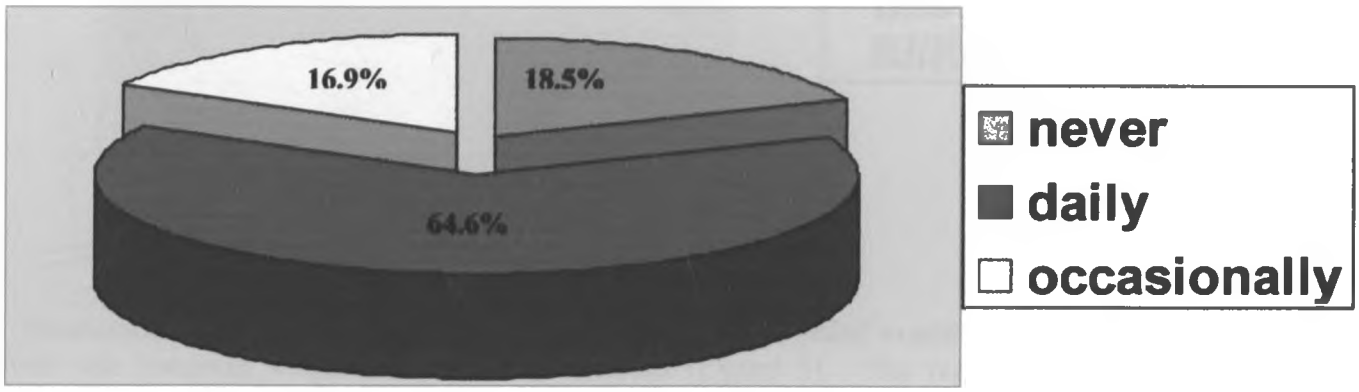
Characteristics	Total N=65	Percentage
Mean age in years ± SD	10.9 ± 4.5	-
Median duration of diabetes in years (IQR)	2(1-4)	-
Sex		
Female (N)	34	52.3
Male (N)	31	47.7
Family history of diabetes		
No (N)	37	56.9
Yes (N)	28	43.1
HbA_{1c} levels		
8% or less (N)	33	50.8
Greater than 8% (N)	32	49.2
Caretaker's level of formal education (Mean number of years ± SD)	11.28 ± 3.1	-
Median monthly household income (IQR)	KES 11000(6000-20000)	-
BMI for age (CDC charts 2000)		
Underweight (N)	11	16.9
Healthy (N)	47	72.3
At risk of being overweight (N)	5	7.7
Overweight (N)	2	3.1

Practices Related to Diabetes Management

The practices related to management of diabetes among study participants including blood glucose monitoring, frequency and doses of insulin administered, and attendance of diabetic clinic varied. Fourty-six (70.8%) of the subjects attended the diabetic clinic monthly while 29.2% attended the clinic less frequently.

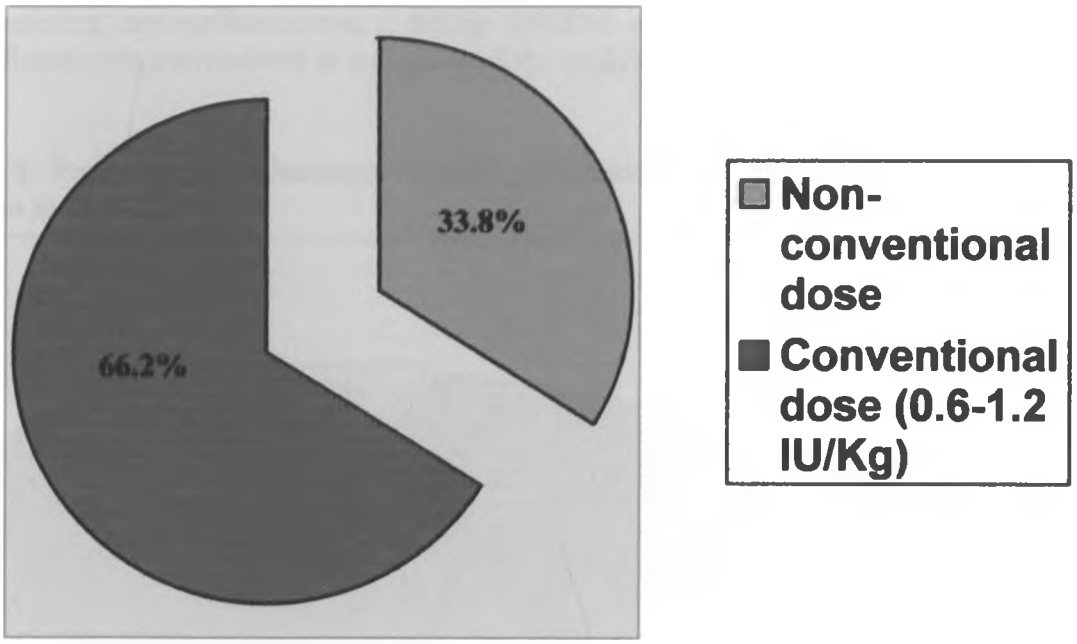
Figure 3 presents the frequency of blood glucose monitoring at home among the 65 children studied. Forty-two (64.6%) subjects monitored their blood glucose levels daily, while 12 (18.5%) never monitored their blood glucose levels at home. Eleven participants monitored their blood glucose levels occasionally.

Figure 3. Frequency of home blood glucose montoring among study participants



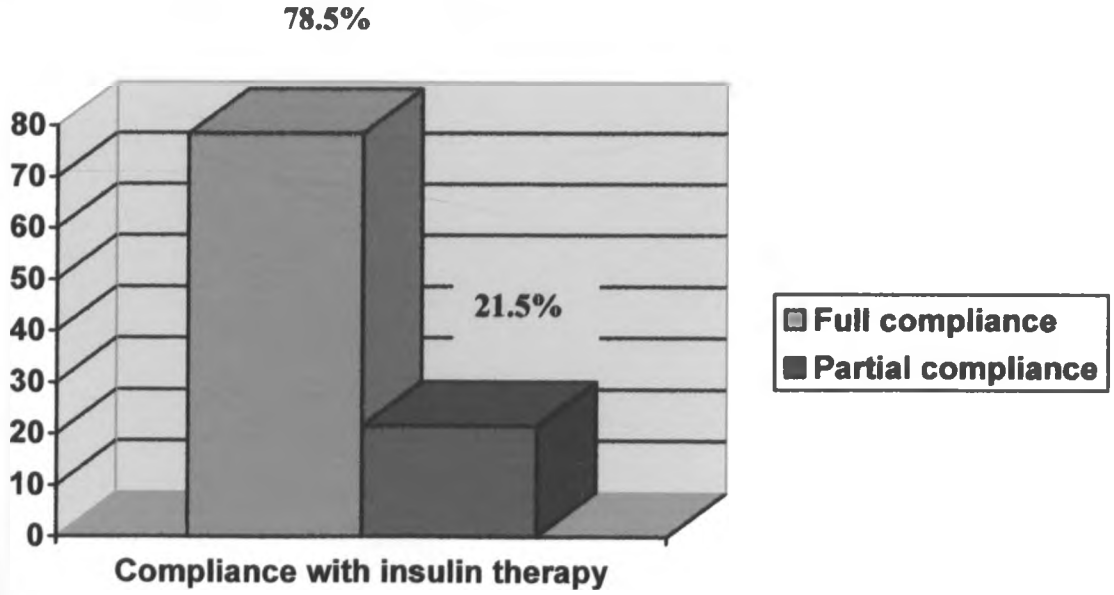
As shown in Figure 4, approximately one in every three insulin prescriptions was higher or lower than the conventional daily dose of 0.6-1.2 IU/Kg.

Figure 4. Daily insulin dose administered



Fourteen (21.5%) of the participants reported that they had failed to administer insulin on at least one occasion within the previous 3 months (Figure 5). The reasons for failing to administer insulin included inadequate knowledge about diabetes and its management, hypoglycemic episodes, forgetting to carry the required dose while away from home and refusal to take insulin.

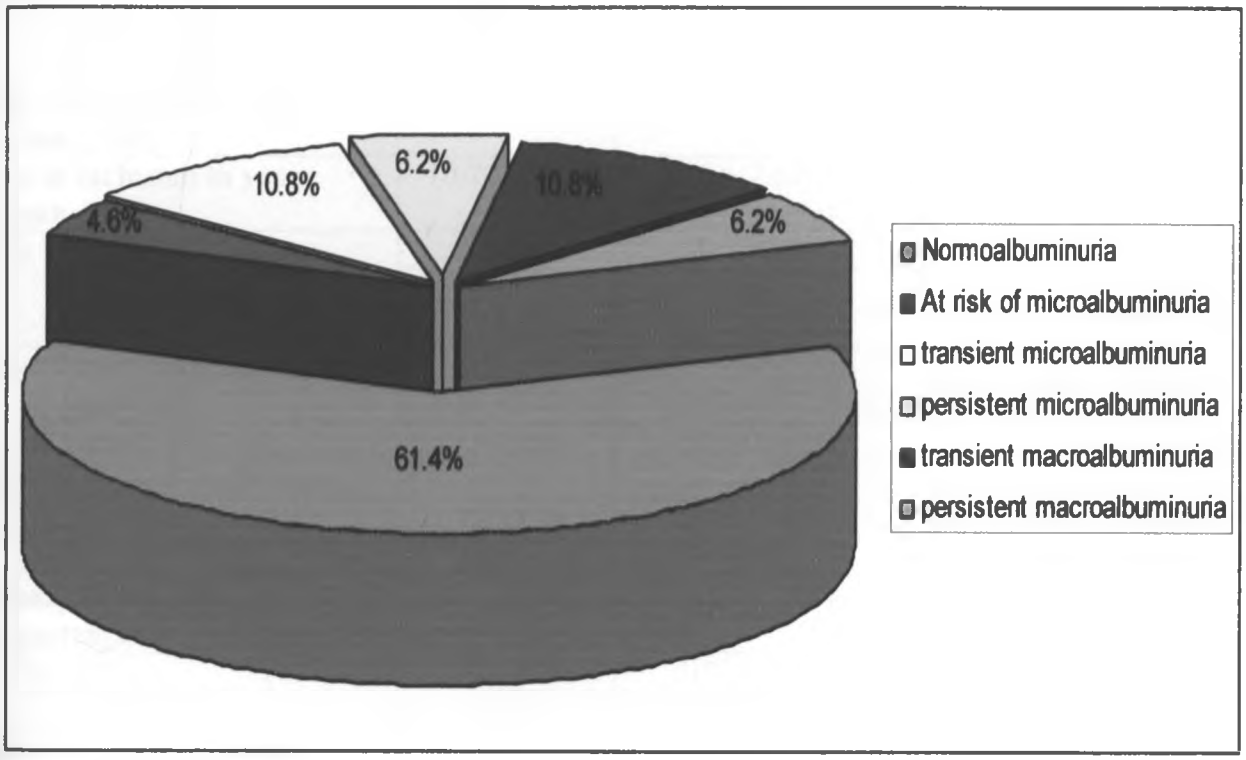
Figure 5. Compliance with insulin therapy in the previous 3 months



The Prevalence of Albuminuria

The prevalence of persistent microalbuminuria (urine albumin to creatinine ratio of 30-300μg/mg on at least two occasions) among diabetic children and adolescents in this study was 6.2% (4 subjects). Three (4.6%) subjects were at risk of developing microalbuminuria (urine albumin to creatinine ratio of 20-29μg/mg on at least two occasions) while 4 (6.2%) had persistent macroalbuminuria. Seven (10.8%) of the study subjects had transient microalbuminuria (normalized at end-point of the study) (Figure 6).

Figure 6. Prevalence of albuminuria among 65 children and adolescents with type 1 diabetes mellitus



BIVARIATE ANALYSIS

SOCIODEMOGRAPHIC CHARACTERISTICS AND PERSISTENT MICROALBUMINURIA

Table 6.1. Association between sociodemographic characteristics and persistent microalbuminuria

Variable	Microalbuminuria		Odds Ratio/ Difference* (95% CI)	Yates chi	P value
	Absent (N=57)	Present (N=4)			
Gender					
Male N(%)	25(43.9)	3(75.0)	0.26(0.03-2.66)	0.48	0.49
Female N(%)	32(56.1)	1(25.0)			
Age at diagnosis in years (Mean ± SD)	8.17 ± 4.56	9.69 ± 5.07	1.52(-3.23-6.27)*	-	0.52
Age at inclusion in years (Mean ± SD)	10.72 ± 4.66	13.69 ± 2.07	-2.94(-7.66-1.78)*	-	0.22
Age at inclusion					
<10 years N(%)	23(40.4)	0(0.0)	-	1.158	0.282
≥10 years N(%)	34(59.6)	4(100.0)			
Duration of diabetes					
<5 years N(%)	46(80.7)	3(75.0)	1.39(0.13-14.72)	0.139	0.709
≥5 years N(%)	11(19.3)	1(25.0)			
Family history of diabetes					
No N(%)	33(57.9)	2(50.0)	1.37(0.18-10.46)	0.046	0.830
Yes N(%)	24(42.1)	2(50.0)			
Family history of hypertension					
No N(%)	38(66.7)	3(75.0)	0.67(0.06-6.85)	0.043	0.836
Yes N(%)	19(33.3)	1(25.0)			
Family history of renal disease					
No N(%)	54(94.7)	3(75.0)	6.00(0.47-76.41)	0.247	0.619
Yes N(%)	3(5.3)	1(25.0)			
Caretaker's level of formal education (Mean ± SD number of completed years)	11.16 ± 3.27	12 ± 0.82	-0.83(-4.13-2.47)*	-	0.62
Median monthly household income in KES (IQR)	11000 (7000-20000)	6500 (8000-22500)	11192(-30811.38-53195.38)*	-	0.37

Gender

Three of the 4 subjects who had persistent microalbuminuria were males. Among the subjects without persistent microalbuminuria there were 32(56.14%) females and 25(43.86%) males. The odds of detecting microalbuminuria among females was 0.26 of the odds among males. There was no statistically significant association between gender and persistent microalbuminuria (p=0.49).

Age at diagnosis

The mean age at diagnosis was 8.17 for the microalbuminuric group and 9.69 for the non-microalbuminuric group. There was no significant difference in the mean age at diagnosis between the two groups (student t test, p=0.52).

Age at inclusion

The microalbuminuric group had a higher mean age at inclusion (student t-test, p value 0.22). All the subjects with persistent microalbuminuria were aged 10 years and above (p=0.282). There was, however, no statistically significant association between the age at inclusion and the occurrence of persistent microalbuminuria.

Duration of diabetes illness

Three of the 4 children with persistent microalbuminuria had had the disease for less than 5 years. There was no statistical association between the duration of diabetes and the presence of persistent microalbuminuria (p=0.709).

Family history of diabetes, hypertension and renal disease

Family history of renal disease was less commonly reported compared to a history of hypertension or diabetes. Four children had a family history of renal disease, 23 had a family history of hypertension and 28 had a family history of diabetes. One (25%) participant with persistent microalbuminuria reported a family history of renal disease, while another had a family history of hypertension. Two (50%) of the microalbuminuric subjects had a family history of diabetes. There was no statistically significant association with the occurence of persistent microalbuminuria.

Caretakers' level of formal education

The mean number of completed years of formal education for the caretakers of the patients with microalbuminuria was 12, while for the non-microalbuminuric group it was 11.16. There was no significant difference in the caretakers' level of formal education between the two groups (student t test, p=0.62).

Economic status

The microalbuminuric group had a lower mean monthly household income (KES 6500) compared to the non-microalbuminuric group (KES 11000). This difference was, however, not statistically significant (Mann-Whitney U test, p=0.37).

Body Mass Index

Table 6.2. Association between body mass index and persistent microalbuminuria

BMI for Age (CDC 2000)	Non-microalbuminuric (N=57)	Microalbuminuric (N=4)	Yates chi	p value
Underweight N(%)	8(14.04)	0(0)	1.29	0.73
Healthy N(%)	42(73.68)	4(100.00)		
At risk of being overweight N(%)	5(8.77)	0(0)		
Overweight N(%)	3(3.51)	0(0)		

Based on the CDC BMI for Age Growth Charts (2000), all the children with persistent microalbuminuria and only 73.7% of the non-microalbuminuric subjects had a healthy body mass index. There was no association between body mass index and the presence of persistent microalbuminuria (p=0.73).

GLYCEMIC CONTROL AND PERSISTENT MICROALBUMINURIA

Table 7. Correlation between glycemic control and persistent microalbuminuria

Variable	Microalbuminuria		Odds Ratio (95% CI)	Yates chi	p value
	Absent (N=57)	Present (N=4)			
HbA_{1c}					
>8% N(%)	29(50.9)	1(25.0)	3.12(0.29-32.9)	0.234	0.63
≤8% N(%)	28(49.1)	3(75.0)			
Hypoglycemia					
No episode N(%)	38(68.4)	3(75.0)	0.72(0.07-7.59)	0.08	0.76
≥1 episode N(%)	18(31.6)	1(25.0)			

Twenty-five percent of the microalbuminuric patients had poor glycemic control while 50.9% of the non-microalbuminuric group had poor glycemic control based on the HbA_{1c} level (p=0.63). Eighteen (31.6%) of the non-microalbuminuric children reported at least one episode of hypoglycemia in the previous 2 weeks compared to only 1 (25%) of children with microalbuminuria (p=0.76). There was no significant association between the occurence of persistent microalbuminuria and glycemic control.

Table 8. Association between knowledge and practices and persistent microalbuminuria

Variable	Microalbuminuria		Odds Ratio (95% CI)	Yates chi	p value
	Absent (N=57)	Present (N=4)			
Knowledge					
Adequate N(%)	47(82.5)	3(75.0)	0.64(0.06-6.79)	0.089	0.765
Inadequate N(%)	10(17.5)	1(25.0)			
Insulin dose					
Low N(%)	16(28.1)	0(0.0)	-	0.417	0.518
Normal or high N(%)	41(71.9)	4(100.0)			
Insulin administration					
Fully compliant N(%)	46(80.7)	2(50.0)	4.18(0.53-33.05)	0.669	0.413
Partially compliant N(%)	11(19.3)	2(50.0)			

Seventy-five percent of microalbuminuric subjects and 82.46% of non-microalbuminuric subjects could identify the signs of hypoglycemia and hyperglycemia and knew what action to take in case of a hyperglycemic or hypoglycemic episode (p=0.765). All the microalbuminuric patients used normal (0.6-1.2 IU/Kg) or high dosages of insulin compared to only 71.9% of the non-microalbuminuric group (p=0.34). Based on previous 3 month recall, two (50%) participants with persistent microalbuminuria forgot to administer insulin for periods longer than 3 consecutive days (p=0.413). There were no significant differences in the knowledge and practices between the two groups.

ASSOCIATION BETWEEN OVERT PROTEINURIA AND VARIABLES

Table 9. Correlation between variables and persistent macroalbuminuria (overt proteinuria)

Variable	Macroalbuminuria		Odds Ratio/ Difference* (95% CI)	Yates chi	P value
	Absent (N=61)	Present (N=4)			
Gender					
Male N(%)	28(45.9)	3(75.0)	0.28(0.03-2.87)	0.38	0.54
Female N(%)	33(54.1)	1(25.0)			
Age at diagnosis in years (Mean ± SD)	8.27 ± 4.57	7.83 ± 2.52	0.44(-5.06-4.19)*	-	0.85
Age at inclusion in years (Mean ± SD)	10.94 ± 4.58	11.58 ± 4.75	-0.64(-5.38-4.09)*	-	0.79
Duration of diabetes					
<5 years N(%)	49(80.3)	3(75.0)	1.36(0.13-14.27)	0.150	0.699
≥5 years N(%)	12(19.7)	1(25.0)			
Family history of diabetes					
No N(%)	35(57.4)	2(50.0)	1.35(0.18-10.19)	0.054	0.816
Yes N(%)	26(42.6)	2(50.0)			
Family history of hypertension					
No N(%)	41(67.2)	1(25.0)	6.15(0.60-62.92)	1.371	0.242
Yes N(%)	20(32.8)	3(75.0)			
Family history of renal disease					
No N(%)	57(93.4)	4(100.0)	-	0.297	0.586
Yes N(%)	4(6.6)	0(0.0)			
HbA_{1c}					
>8% N(%)	30(49.2)	3(75.0)	0.32(0.03-3.40)	0.235	0.628
≤8% N(%)	31(50.8)	1(25.0)			
Hypoglycemia					
No episode N(%)	42(68.9)	1(25.0)	6.63(0.60-73.52)	1.56	0.21
≥1 episode N(%)	19(31.6)	3(75.0)			
Knowledge					
Adequate N(%)	50(82.0)	3(75.0)	0.66(0.06-6.96)	0.101	0.751
Inadequate N(%)	11(18.0)	1(25.0)			
Insulin dose					
Low N(%)	16(26.2)	1(25.0)	1.07(0.10-11.22)	0.284	0.594
Normal or high N(%)	45(73.8)	3(75.0)			
Insulin administration					
Fully compliant N(%)	48(78.7)	3(75.0)	1.23(0.12-12.84)	0.206	0.650
Partially compliant N(%)	13(21.3)	1(25.0)			
BMI for Age (CDC 2000)					
Underweight	8(13.11)	2(50.00)	-	2.79	0.43
Healthy	46(75.41)	2(50.00)			
At risk of being overweight	5(8.20)	0(0)			
Overweight	2(3.28)	0(0)			

Three of the 4 subjects with persistent macroalbuminuria were males while only 45.9% of the non-macroalbuminuric subjects were males (p=0.54). There was no significant difference in the mean age at diagnosis between the macroalbuminuric group (7.83 years) and the non-macroalbuminuric group (8.27 years)(student t test, p=0.85). There was also no significant difference in the age at inclusion of subjects into the study between the two groups (student t test, p=0.79). Three of the 4 subjects with persistent overt proteinuria and 80.3% of the non-macroalbuminuric patients had had diabetes for less than 5 years. There was no association between duration of diabetes illness and persistent overt proteinuria (p=0.699).

Three of the 4 subjects with persistent macroalbuminuria and only 32.8% of those in the non-macroalbuminuric group reported a family history of hypertension (p=0.242). Non of the macroalbuminuric patients and only 6.6% of the non-macroalbuminuric patients reported a family history of renal disease (p=0.586). A family history of diabetes was reported by about half of the subjects in both groups (p=0.816). There was no statistical association between family history of diabetes, hypertension or renal disease and the occurence of persistent overt proteinuria.

Three patients with persistent overt proteinuria and half of the non-macroalbuminuric subjects had poor glycemic control based on the HbA_{1c} level (p= 0.628). Nineteen (31.6%) of the non-macroalbuminuric patients and 3 (75%) of the macroalbuminuric subjects reported at least one hypoglycemic episode in the previous 2 weeks (p=0.21). There was, however, no significant association between glycemic control and persistent macroalbuminuria.

Seventy-five percent of the non-macroalbuminuric subjects and only 50% of the macroalbuminuric subjects had a healthy body mass index for age based on the CDC growth charts. This difference was however not statistically significant (p=0.43). There were no significant differences in the knowledge and practices between the subjects in the two groups.

10. DISCUSSION

In this prospective cross-sectional study of 65 youth with type 1 diabetes mellitus on follow up at the Kenyatta National Hospital diabetic outpatient clinic in Nairobi, with a median duration of diabetes of 2 years, the prevalence of persistent microalbuminuria was 6.2%. There are few studies from sub-Saharan Africa dealing with microalbuminuria in children. This prevalence was relatively low compared with prevalences found in other studies in sub-Saharan Africa. Lutale et al[24] in Dar es Salaam, Tanzania, found a prevalence of 12% among type 1 diabetic patients with a median duration of diabetes of 3 years and a mean age of 21 (4-44.8) years. In a survey on acute and chronic complications in children and adolescents aged 5 to 18 years (mean ± SD age 12.6 ± 3.5), with a mean duration of diabetes of 4.76 years, Majaliwa et al[27] in Tanzania reported a prevalence of 29.3%. Differences in ethnic groups, methodology, mean age of the study population and definition of microalbuminuria may account for these differences in the prevalences. Genetic factors are believed to be responsible for the development of diabetic nephropathy. In the current study, the urine albumin-to-creatinine ratio was used to determine the presence of microalbuminuria. This method does not account for patient weight or muscle mass and may therefore overestimate or underestimate the prevalence of microalbuminuria. Microalbuminuria was assessed on at least two occasions within a 3 to 6 month period in this study. In some of the other studies from sub-Saharan Africa, this assessment was done on only one occasion, which may have resulted in misclassification bias as microalbuminuria may be transient in upto 50% of patients.[22] The mean age in this population was 10.9 ± 4.5 years which was lower than that of the population in the study by Lutale et al[24] and Majaliwa et al.[27] Some studies have demonstrated that increasing age is associated with a higher risk for the development of microalbuminuria.[12,22,23]

Our data are similar to those of a study done in France by the GECER Study Group,[21] who found that the proportion of persistent microalbuminuria was 5.1 ± 1.6%. This was a multicentre cross-sectional survey including 702 children and adolescents (mean ± SD age 14.3 ± 2.9 years) with type 1 diabetes duration of 7.6 ± 3.1 years. Similarly, Bruno et al[33] reported a low (7%) prevalence of microalbuminuria in young Italian insulin-dependent diabetic patients with a duration of diabetes of 3 to 5 years. In a longitudinal study by Donaghue et al[22] in Australia, where 972 youth were followed up for upto 15 years, the incidence of persistent microalbuminuria was 4.6 per 1,000 patient-years. These patients had a median duration of diabetes of 6.5 years. In a longitudinal study in the UK, Schultz et al[15] found a prevalence of 4.8% of persistent microalbuminuria in children with type 1 diabetes. Estimates of the prevalence of microalbuminuria in childhood in various other reports vary between 9.7 to 23.1% (Table 2). This wide range may be due to, in addition to the factors mentioned above, differences in population size and length of follow up.

In the current study, 4.6% of the patients were found to be at risk of developing microalbuminuria. Donaghue et al[22] demonstrated that children and adolescents with borderline microalbuminuria were more than twice as likely to develop persistent microalbuminuria even after adjusting for duration of diabetes. This finding has been documented in other studies.[34,35] This suggests that early intervention at a lower level of albuminuria than recommended by current guidelines may be warranted. Among all the children with microalbuminuria in the current study, 63.6% were transient. This was higher than reported by Donaghue et al[22] who found that 48.4% of cases of microalbuminuria were transient. The transient nature of microalbuminuria in children and adolescents would suggest the need for at least 12 months of documented microalbuminuria before treatment is considered. Persistent overt proteinuria was found in 6.2% of the patients in this study. This was higher than those of the studies by Lutale et al[24] (1.1%) and Zahra et al[28] (1%).

GENDER

Three out of the 4 children with persistent microalbuminuria in this study were males, and this finding was similar for those with persistent overt proteinuria. This finding was, however, not statistically significant. This is consistent with data from Mullis et al[36] in a study done in Switzerland among 127 children and adolescents aged 3 to 21 years, where they documented that microalbuminuria was not strictly dependent on gender. In contrast, the MIDAC[9] research groups, as well as various other studies[11,15,25] found that the development of microalbuminuria was accelerated in girls.

AGE AT INCLUSION

The mean age of the patients with microalbuminuria was higher than that of the non-microalbuminuric patients, although not significantly different. This is in agreement with Lutale et al, [24] who found no significant differences in age at inclusion between patients with normoalbuminuria and those with abnormal albumin excretion rates. In contrast, Rudberg et al,[12] Donaghue et al[22] and Moayeri et al[23] demonstrated that older age was associated with microalbuminuria.

AGE AT DIAGNOSIS

There was no significant difference in the age at diagnosis between the microalbuminuric and non-microalbuminuric patients. This finding was similar to that found in a longitudinal evaluation of urinary albumin excretion in 118 children with type 1 diabetes attending a single clinic over a period of 7 years in Tehran, Iran, by Moayeri et al.[23] On the other hand, the study done by Gallego et al[25] in Australia indicated that children diagnosed with type 1 diabetes at younger ages had a prolonged time for developing microalbuminuria. This was after evaluation of 955 type 1 diabetic children with a mean diabetes duration of 7.6 years and a mean age at onset of diabetes of 8.5 years. In a longitudinal analysis, Donaghue et al[22] also observed that increasing age at diagnosis increased the risk of microalbuminuria.

DURATION OF DIABETES

The American Diabetes Association recommends annual screening for the presence of microalbuminuria in type 1 diabetic patients who have had diabetes for more than 5 years and all type 2 diabetic patients starting at diagnosis. Previous studies have found that diabetes duration is a major factor predisposing to the development of microalbuminuria. Zahra et al[28] in a study in Hamedan, Iran, reported that microalbuminuria increased with increasing disease duration. This data was similar to that of Moayeri et al,[23] Gallego et al[25] and the GECER study group.[21] In contrast, Majaliwa et al[27] found no correlation between duration of diabetes and the occurrence of microalbuminuria. Rudberg et al[12] in a study done in Sweden documented that 6 out of 17 children had an onset of microalbuminuria before 5 years of diabetes. Similarly, we observed that 3 out of the 4 children with persistent microalbuminuria had a diabetes duration of less than 5 years. These findings suggest that screening for microalbuminuria in children and adolescents with type 1 diabetes should start at diagnosis rather than after 5 years duration as recommended by the American Diabetes Association.

PUBERTY

In this study we observed that all the patients with persistent microalbuminuria were adolescent. In fact, the youngest patient was 12 years at inclusion into the study. Three of the four children with persistent microalbuminuria were pubertal based on the Tanner sexual maturity rating. Previous studies[10,20] have indicated that the onset of microalbuminuria before puberty occurs only rarely. Moayeri et al[23] found that the onset of microalbuminuria in 22 of 23 children was after puberty and in one patient it occurred before puberty. The incidence of microalbuminuria in type 1 diabetes, thus, increases at puberty,[12] a time of exaggerated physiological insulin resistance.[37-39] Higher androgen and growth hormone levels have been found in adolescents with type 1 diabetes in association with higher albumin excretion.[40,41]

BLOOD PRESSURE

The association between microalbuminuria and hypertension in childhood has been demonstrated in some studies but not in others. Mathiesen et al,[20] Zahra et al[28] and Moayeri et al[23] demonstrated that microalbuminuria was significantly associated with hypertension. On the other hand, Lutale et al[24] found no significant differences when the systolic and diastolic blood pressures of type 1 diabetic patients with normal albumin excretion rates were compared with those with abnormal albumin excretion rates. This was in agreement with the MIDAC study group[9] who found no association between microalbuminuria and hypertension. All the children in the current study had a normal blood pressure for age, except for one child with persistent overt proteinuria who had an elevated systolic and diastolic blood pressure. Although hypertension may aggravate the renal disease, it does not seem to be a prerequisite for the development of microalbuminuria. Twenty-four hour blood pressure monitoring would, however, be more reliable than simply measuring the blood pressure in the clinic.

BODY MASS INDEX (BMI)

Based on the Centre for Disease Control and Prevention 2000 BMI for age charts, and in agreement with Lutale et al[24] and the MIDAC group, [9] our study found no significant association between the BMI and the occurrence of persistent microalbuminuria. In contrast, Donaghue et al[22] documented that obesity was associated with the development of persistent microalbuminuria.

GLYCEMIC CONTROL

In this study, we found that 50.9% of the children and adolescents with type 1 diabetes had poor glycemic control. This was an improvement compared to 2003 when Ngwiri et al found that 72% of the children had poor glycemic control. Better contact between the patients and the health care providers, as a result of more frequent clinic visits since the introduction of the paediatric endocrine clinic, may explain this improvement. In addition, each of the patients is able to get in touch with a health care worker by phone. Poor glycemic control is a well-defined contributor to the development and progression of microalbuminuria.[15,22,23,25,28] However, the current study, as well as most of the available studies from sub-Saharan Africa did not demonstrate any significant relationship between the level of glycemic control and the development of microalbuminuria.[24,36]

INSULIN DOSE

All the children with persistent microalbuminuria in this study received a conventional dose of insulin (0.6-1.2 IU/Kg/day). There was no significant association with insulin dose, contrary to the findings in the study by Zahra et al,[28] where the frequency of microalbuminuria was noted to be significantly higher in patients taking lower doses of insulin corrected to their body weight.

FAMILY HISTORY OF DISEASE

We documented no significant associations between family history of diabetes, hypertension or renal disease and the presence of persistent microalbuminuria. The GECER study group, [21] in contrast, reported that the presence of maternal hypertension was an independent risk factor for microalbuminuria.

KNOWLEDGE AND ECONOMIC STATUS

There were no significant differences in the level of knowledge around diabetes among the microalbuminuric and the non-microalbuminuric group. The subjects with persistent microalbuminuria had a lower median household monthly income compared to the non-microalbuminuric group. Considering the reasonable glycemic control among the microalbuminuric patients, the significance of this is unclear from this study. The author is not aware of any studies that have assessed the impact of knowledge and socioeconomic status on the occurrence of microalbuminuria.

11. STUDY LIMITATIONS

- Some patients may have had undiagnosed sickle cell disease or orthostatic proteinuria, and may thus have been mistaken to have diabetes-related renal disease.
- It is not easy to obtain a pure midstream specimen of urine in young children who are not toilet-trained. For these children urine was collected using a plastic urine bag that sticks onto the skin. This may have resulted in contamination of the urine with microbes from the skin, with resultant proliferation and loss of albumin.
- This study was underpowered due to the limited number of type 1 diabetic patients and the low prevalence of microalbuminuria. This may account for the lack of statistical correlation to the commonly acknowledged risk factors for microalbuminuria.
- The study was done in a relatively short period. Further studies with a longer period of follow up may show different results.

12. CONCLUSIONS

- The prevalence of persistent microalbuminuria in children and adolescents with type 1 diabetes mellitus on follow up at the outpatient clinic in Kenyatta National Hospital is 6.2%. These children may be at particular risk of cardiovascular morbidity and mortality, and later end-stage renal disease. This study detects, however, a much lower prevalence than reported in previous studies from sub-Saharan Africa.
- There were no significant differences in sociodemographic characteristics and glycemic control among the microalbuminuric and non-microalbuminuric patients.
- Three of the four children with persistent microalbuminuria were pubertal based on the Tanner sexual maturity rating, suggesting the influence of endocrine changes during this period.
- Three of the four subjects with persistent microalbuminuria had had diabetes for less than 5 years, and thus had an early onset of microalbumuninuria.

13. RECOMMENDATIONS

- A longitudinal study should be carried out with a higher number of patients followed over time. This may also provide information on the natural history of microalbuminuria in children with type 1 diabetes mellitus. It is also recommended that glomerular filtration patterns be assessed in these children, which was beyond the scope of this study.
- Screening for microalbuminuria in patients with type 1 diabetes mellitus should begin at the time of diagnosis of diabetes rather than after the recommended five years.

REFERENCES

1. IDF. Incidence of diabetes. *Diabetes Atlas 2006*: 2. http://www.eatlas.idf.org/incidence/.
2. Swai A. B. M., Prospective Study of Incidence of Juvenile Diabetes Mellitus over 10 Years in Dar es Salaam, Tanzania. *British Medical Journal.* 1993; 306: 1570–72.
3. Elamin A., Omer M. I., Zein K., Tuvemo T. Epidemiology of Childhood Type I Diabetes in Sudan, 1987–1990. *Diabetes Care.* 1992; 15: 1556–59.
4. Afoke A. O., Ejeh N. M., Nwonu E. N., Okafor C. O., Udeh N. J., Ludvigsson J. Prevalence and Clinical Picture of IDDM in Nigerian Ibo School Children. *Diabetes Care.* 1992; 15: 1310–12.
5. ISPAD Clinical Practice Consensus Guidelines 2006–2007. Definition, epidemiology and classification. *Pediatric Diabetes* 2006: 7: 343–351.
6. Richard E. Behrman et al. *Nelson Textbook of Pediatrics.* 17th ed. 2004; 1947-1972.
7. ISPAD Clinical Practice Consensus Guidelines 2006–2007. *Pediatric Diabetes* 2007: 8: 163–170.
8. Gill G. V., Huddle K. R., Rolfe M. Mortality and Outcome of Insulin-Dependent Diabetes in Soweto, South Africa. *Diabetic Medicine.* 1995; 12: 546–50.
9. Moore TH, Shield JP. Prevalence of abnormal urinary albumin excretion in adolescents and children with insulin dependent diabetes: the MIDAC study. Microalbuminuria in Diabetic Adolescents and Children (MIDAC) research group. *Arch Dis Child.* 2000 Sep; 83(3): 239-243.
10. Mortensen HB, Marinelli K, Norgaard K, Main K, Kastrup KW, Ibsen KK, Villumsen J, Parving HH. A nation-wide cross-sectional study of urinary albumin excretion rate, arterial blood pressure and blood glucose control in Danish children with type 1 diabetes mellitus. Danish Study Group of Diabetes in Childhood. *Diabet Med.* 1990 Dec; 7(10):887-897.
11. Holl RW, Grabert M, Thon A, Heinze E. Urinary excretion of albumin in adolescents with type 1 diabetes: persistent versus intermittent microalbuminuria and relationship to duration of diabetes, sex, and metabolic control. *Diabetes Care.* 1999 Sep; 22(9):1555-1560.
12. Rudberg S, Ullman E, Dahlquist G. Relationship between early metabolic control and the development of microalbuminuria--a longitudinal study in children with type 1 (insulin dependent) diabetes mellitus. *Diabetologia.* 1993 Dec; 36(12):1309-1314.
13. Davies AG, Price DA, Postlethwaite RJ, Addison GM, Burn JL, Fielding BA. Renal function in diabetes mellitus. *Arch Dis Child.* 1985 Apr; 60(4):299-304.
14. Rowe DJ, Hayward M, Bagga H, Betts P. Effect of glycaemic control and duration of disease on overnight albumin excretion in diabetic children. *Br Med J (Clin Res Ed).* 1984 Oct 13; 289(6450):957-959.
15. CJ Schultz, T Konopelska-Bahu, RN Dalton, TA Carroll, I Stratton, EA Gale, A Neil and DB Dunger. Microalbuminuria prevalence varies with age, sex, and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study. Oxford Regional Prospective Study Group. *Diabetes Care*, Vol 22, Issue 3; 495-502.
16. Barkai L, Vamosi I, Lukacs K. Enhanced progression of urinary albumin excretion in IDDM during puberty. *Diabetes Care* 1998; 21:1019–23.
17. DeClue TJ, Campos A. Diabetic nephropathy in a prepubertal diabetic female. *J Pediatr Endocrinol.* 1994 Jan-Mar; 7(1):43-46.
18. Bayazit AK, Yuksel B, Noyan A, Onenli N, Gonlusen G, Ozer G, Anarat A. Early onset of diabetic nephropathy in a child with type 1 diabetes mellitus. *Turk J Pediatr.* 2003 Jan-Mar; 45(1):55-58.

19. Jones CA, Leese GP, Kerr S, Bestwick K, Isherwood DI, Vora JP, Hughes DA, Smith C. Development and progression of microalbuminuria in a clinic sample of patients with insulin dependent diabetes mellitus. *Arch Dis Child.* 1998 Jun; 78(6):518-523.
20. ER. Mathiesen, N Saurbrey, E Hommel, HH Parving. Prevalence of microalbuminuria in children with Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, Vol. 29, No. 9, pg. 640-643 (September).
21. Lévy-Marchal C, Sahler C, Cahané M, Czernichow P; GECER Study Group. Risk factors for microalbuminuria in children and adolescents with type 1 diabetes. *J Pediatr Endocrinol Metab.* 2000 Jun;13(6):613-20.
22. ML Stone, ME Craig, AK Chan, JW Lee, CF Verge, KC Donaghue. Natural History and Risk Factors for Microalbuminuria in Adolescents With Type 1 Diabetes. Diabetes Care 2006; 29:2072-2077.
23. H Moayeri, H Dalili. Prevalence of microalbuminuria in children and adolescents with diabetes mellitus type 1. *Acta medica iranica*, 44(2): 105-110; 2006.
24. JJ Kachuchuru Lutale, H Thordarson, ZG Abbas, K Vetvik. Microalbuminuria among Type 1 and Type 2 diabetic patients of African origin in Dar Es Salaam, Tanzania. *BMC Nephrology* 2007; 8:2 doi:10.1186/1471-2369-8-2.
25. Gallego, P Herold, Bulsara, M Kishor, Frazer, Fiona, Lafferty, A Richard, Davis, E Ann, Jones, T William. Prevalence and risk factors for microalbuminuria in a population-based sample of children and adolescents with T1DM in Western Australia. *Pediatric Diabetes*, Vol.7, No.3, June 2006, pp.165-172(8).
26. Abdeyazdan Z, Hashemipour M, Hasanzadeh A, Pour Naghshban Z, Kabirzadeh M. Microalbuminuria in Type 1 Diabetes Mellitus. *Shiraz E-Medical Journal* Vol. 8, No.1, January 2007.
27. ES Majaliw.a, E Munubhi, K Ramaiya, R Mpembeni, A Sanyiwa, A Mohn, F Chiarelli. Survey on Acute and Chronic Complications in Children and Adolescents With Type 1 Diabetes at Muhimbili National Hospital in Dar es Salaam, Tanzania. *Diabetes Care* 30:2187-2192, 2007.
28. Zahra R, Hossein EM, Somayeh S. Frequency of Microalbuminuria in Type 1 Diabetic Children. *Iran J Pediatr*, Dec 2009; Vol. 19 (No 4), Pp:404-408.
29. Defronzo RA. Diabetic nephropathy. In: Porte D, Sherwin R, eds. *Ellenberg and Rifklin's diabetes mellitus*. Sixth edition. New York: Mc Graw-Hill Companies; 2002. p. 723-740.
30. Vats A, Derubertis F. Diabetic nephropathy. In: Sperling MA, editor. *Type I diabetes, etiology and treatment*. 1st ed. Totowa, NJ: Human Press Inc; 2003. p. 409-427.
31. Francis J, Rose SJ, Raafat F, Milford DV. Early onset of diabetic nephropathy. *Arch Dis Child.* 1997 Dec; 77(6):524-525.
32. Balasubramanyam A, Zern JW, Hyman DJ, Pavlik V. New profiles of diabetic ketoacidosis: type 1 vs type 2 diabetics and the effect of ethnicity. *Arch Int Med* 1999; 159:2317-2322.
33. G Bruno, G Pagano. Low Prevalence of Microalbuminuria in Young Italian Insulin-dependent Diabetic Patients with Short Duration of Disease: a Population-based Study. *Diabetic Medicine*, Vol. 13, No 10, July 2004, pp. 889-893.
34. Couper JJ, Clarke CF, Byrne GC, Jones TW, Donaghue KC, Nairn J, Boyce D, Russell M, Stephens M, Raymond J, Bates DJ, McCaul K: Progression of borderline increases in albuminuria in adolescents with insulin-dependent diabetes mellitus.*Diabet Med* 14: 766–771, 1997.
35. Schultz CJ, Neil HA, Dalton RN, Dunger DB: Risk of nephropathy can be detected before the onset of microalbuminuria during the early years after diagnosis in type 1 diabetes. *Diabetes Care* 23: 1811–1825, 2000.

36. P Mullis, HP Köchli, K Zuppinger, HP Schwarz. Intermittent microalbuminuria in children with type 1 diabetes mellitus without clinical evidence of nephropathy. European *Journal of Pediatrics*, Vol. 147, No 4, May 1988, pp. 385-388.
37. Osberg I, Peter Chase H, Garg SK. , et al. Effects of storage time end temperature on Measurement of small concentrations of albumin in urine. *Clin Chem*, 1990;36:1428-1430.
38. Shield JPH, Hunt LP, Morgan JE, Pennock CA. Are frozen urine samples acceptable for estimating albumin excretion in research? *Diabet Med* 1995;12:713-716.
39. Lloyd DR, Hindle EJ, Marples J, Gatt JA. Urinary albumin measurement by immunoturbidimetry. *Ann Clin Chem* 1987;24:209-210.
40. McLauchlan DM. Creatinine, urate and urea. In: *Gowenlock AH, ed. Varley's practical clinical chemistry., 6th ed. London: Heinemann Medical Books*, 1988;305-367.
41. de Man SA, Andre JL, Bachmann H, et al. Blood pressure in child hood: pooled findings of six European studies. *J Hypertens* 1991;9:109-114.

PATIENT QUESTIONNAIRE

Study No.....
Name:.....
Hospital No.....
Place of work of parent/guardian.....
Contact details: P.O. Box.....
Tel. No.....

Date of birth (dd/mm/yy) ☐☐ ☐☐ ☐☐

Age: ☐☐ Years ☐☐ Months
Weight.....kg (to nearest 50g) Height.....cm (to nearest 5mm)
Blood Pressure.....mmHg
Sexual Maturity Rating (prepubertal or pubertal).....
Year of diagnosis of diabetes mellitus.....

Sociodemographic Characteristics

1. Gender ☐ Male ☐ Female
2. Usual residence.....
3. Patient's level of education

☐ Primary school Number of completed years.....

☐ Secondary school Number of completed years.....

☐ Tertiary level Number of completed years .....

☐ None ☐ Other (specify).....

4. Level of education of parent/guardian

☐ Primary school Number of completed years .....

☐ Secondary school Number of completed years .....

☐ Tertiary level Number of completed years .....

☐ None ☐ Other (specify).....

5. Parent/guardian's monthly income (Kshs).....

6. Financial assistance ☐ Relatives ☐ Employer ☐ Diabetic clinic

☐ Non-governmental Organization ☐ None ☐ Other (specify).....

Medical History

1. How often do you attend the diabetic clinic?

☐ Monthy ☐ every 2-3 months ☐ every 4-5 months ☐ ≥ 6 monthly

2. Number of admissions in the past 3 months.....

Reasons for admissions ☐ Hypoglycemia ☐ Hyperglycemia ☐ Sepsis

☐ Diabetic ketoacidosis ☐ Other (specify).....

3. How frequently do you monitor blood glucose levels at home?

☐ daily ☐ ≥ once weekly ☐ ≥ once monthly ☐ ≤ once monthly ☐ Never

If never, what hinders you from doing so?

☐ not aware about home monitoring ☐ donot have a glucometer

☐ other (specify).....

4. What are the symptoms of hypoglycemia? ☐ Anxiety ☐ Sweating ☐ Hunger

☐ Awareness of heartbeat ☐ Tremor ☐ Weakness ☐ Dizziness ☐ Nausea

☐ Headache ☐ Personality changes ☐ Inability to concentrate ☐ Confusion

☐ Visual disturbances ☐ Convulsion ☐ Paraesthesia ☐ Slurred speech

☐ Donot know ☐ Other (specify).....

5. How many hypoglycemic episodes have you had in the past 2 weeks?

☐ None ☐ One ☐ Two ☐ Three or more

6. What do you do when blood glucose is low? ☐ Give sugar-containing foods

☐ Donot know ☐ Other (specify).....

7. What are the symptoms of hyperglycemia? ☐ Polyuria ☐ Polydipsia ☐ Polyphagia

☐ Nocturia/ Enuresis ☐ Weight loss ☐ Donot know

☐ Other (specify).....

8. What do you do when blood glucose is high? ☐ Give insulin ☐ Donot know

☐ Other (specify).....

9. Use of home remedies/ herbal medication ☐ Yes ☐ No

If yes, specify.....

10. Dosage of insulin .....IU a.m .....IU noon .....IU p.m .....(IU/kg/day)

11. Missed insulin doses in the last 3 months

☐ Never ☐ Once per week ☐ Twice per week ☐ ≥ 3 consecutive days

Reason for missing dose ☐ Forgot ☐ Ran out of insulin ☐ Could not afford insulin

☐ Other (specify).....

12. Cost of insulin per vial (Kshs.).....

13. Total cost of each clinic visit (including transport, treatment) Kshs.....

14. Number of counseling sessions received on diabetes care

☐ Three or more ☐ Two ☐ One ☐ None

15. What causes diabetes? ☐ Insulin deficiency ☐ Donot know

☐ Other (specify).....

16. Personal history of renal disease ☐ Yes ☐ No

If yes, what is the cause? ☐ Diabetes ☐ Hypertension ☐ Chemotherapy

☐ Other (Specify) .....

17. Family history of diabetes mellitus ☐ Yes ☐ No

If yes, who? ☐ Father ☐ Mother ☐ Sibling

☐ Other (specify).....

18. Family history of kidney disease ☐ Yes ☐ No

If yes, who? ☐ Father ☐ Mother ☐ Sibling

☐ Other (specify).....

19. Family history of hypertension ☐ Yes ☐ No

If yes, who? ☐ Father ☐ Mother ☐ Sibling

☐ Other (specify).....

20. Cigarette smoking ☐ Yes ☐ No

LABORATORY TESTS

1. Urinalysis

(i) ketones..... protein..... nitrites..... leucocytes.....
glucose..... blood..... leucocyte esterase..... pH.....
specific gravity.....

(ii) ketones..... protein..... nitrites..... leucocytes.....
glucose..... blood..... leucocyte esterase..... pH.....
specific gravity.....

(iii) ketones..... protein..... nitrites..... leucocytes.....
glucose..... blood..... leucocyte esterase..... pH.....
specific gravity.....

2. Urine Albumin-to-Creatinine Ratio (mg/g) (i) .....
(ii) .....
(iii) .....

3. HbA_{1c} (%).....

Appendix B

CONSENT FORM

Dear Patient/ Parent/ Guardian,

My name is Dr. Amolo. One of the long term complications of diabetes is kidney disease. A urine test exists that can detect its presence in the early stages by looking for small amounts of protein in the urine (microalbuminuria). I am conducting a research study to find out what proportion of children with diabetes at the Kenyatta National Hospital have this early stage of kidney disease. I would like to include you/ your child as a participant.

This will require that I administer to you a questionnaire, examine and take blood and urine samples from you/ your child. The blood sample will be used to confirm the quality of your/ your child's blood sugar control over the previous three months using a test called Hemoglobin A_{1c} test. This will involve a finger prick which is a minimally invasive procedure. The amount of blood drawn (20µl) is too minimal to affect your child in any adverse way. Only one blood sample will be obtained from you/ your child. Two or three morning specimens of urine of 10ml each will be obtained from you/ your child to determine the presence of protein in urine.

Participation in this study is voluntary and your decision on whether to participate or not will not prejudice your/ your child's care in any way. Strict confidentiality will be observed at all times. In all instances, your primary caregiver will be informed of all the results in view of more stringent follow up and added therapy.

I hope that you accept for yourself/ your child to participate in this study, as its outcome will impact on the future management of diabetes mellitus in our country.

Consent

I Mr/Mrs/Miss..... being a person aged 18 years and over, having read/ been explained to the above, and in the knowledge that it is voluntary, do hereby give consent for myself/ my child to participate in this study.
I understand that I/ my child have the right to withdraw from the research at any time, for any reason, without penalty or harm.

.....
Patient/ Parent/ Guardian's signature
Date:.....

.....
Child's signature if above 7 years (Assent)
Date:.....

.....
Investigator's signature
Date:.....

For any questions/ clarification, contact the principle investigator on:
telephone number: 0722431743
email address: priscaamolo@yahoo.com